D3 selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, threonine and cysteine.

REMARKS

Claims 54-118 are pending in the application. Claims 84-96 and 108-114 have been withdrawn from consideration. Claims 88 and 109 have been amended to correct obvious typographical errors.

New figures 1, 2, 3, and 5 are submitted to address the comments in the Draftsperson's review.

The Invention

Applicants' invention provides methods for altering amino acid compositions of proteins of interest while at least substantially retaining the native conformation of those proteins. The methods make use of interacting molecules which are capable of binding with the native protein and recognizing its native conformation. These interacting molecules include both antibodies and derivatives thereof as well as non-antibody proteins capable of oligomerization and dimerization with the native protein of interest **so long as the object of the invention is achieved, *i.e.*, ascertaining whether the conformation of the protein of interest has been altered** by the changes in amino acid composition.

Claim Rejections Under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

Claims 54-83, 97-107, and 115-118 were rejected under 35 U.S.C. §112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention," as "explained in the previous Office action." (Office Action of 2 January 2002, page 2, #4). The Examiner goes on to repeat the reasons for rejection stated in the previous action, stating that undue experimentation would be required for those of skill in the art

to make and use the invention. This rejection is respectfully traversed.

Applicants respectfully disagree with the assessment in the Office Action. In the previous Response of 16 October 2001, Applicants discussed that an examination of the Wands factors in this case leads to the conclusion that the disclosure fully enables the invention. Applicants reviewed that the specification described the use of oligomerizing proteins and provided a working example of the use of oligomerizing proteins. Applicants also provided copies of a number of scientific publications. Collectively, these scientific publications document that the prior art supports techniques involving the use of antibodies to bind proteins. Thus, Applicants submit that the claims are fully described and enabled by the specification.

However, the Office Action merely repeats the previous grounds for rejection, *i.e.*, that the amount of experimentation required to practice the invention would be undue. In repeating these grounds for rejection, the Office Action does not cite any scientific publications or other authority for support. Applicants remind the Examiner that under 37 C.F.R. §1.110(d)(2), if a rejection is based on the personal knowledge of the Examiner, the Examiner must provide support in the form of an affidavit so that the basis for the rejection may be refuted or explained. Thus, Applicants request that such support be provided or the rejection be withdrawn.

The Office Action states that "every protein has a different structural characteristic such that using antibodies to screen for conformational changes would not necessarily be routine." (Office Action of 2 January 2002, page 3, #6). Applicants wish to emphasize that the present invention is drawn to altering amino acid compositions of proteins of interest while at least **substantially retaining the native conformation** of those proteins. Thus, the immunologically based experiments utilized in the methods of the invention are used to determine whether an altered protein has retained the native conformation, not to determine which of many possible conformations the novel protein has adopted. As discussed in the Rule 132 declaration filed herewith, immunologically-based experiments to determine whether a protein retains the native conformation are readily carried out by one of skill in the art and do not constitute undue experimentation.

Indeed, the Examiner apparently acknowledges that the prior art teaches what is necessary for the practice of the present invention—*i.e.*, that the prior art teaches "the use of antibodies to

discern between completely folded proteins from an identical protein which is not folded to the native conformation." (Office Action of 2 January 2002, page 3, #6). However, the Office Action concludes that "the instant claims encompass using **immunologically based** experiments in the assessment of a protein's native conformation, **an area of work which is not routine** and would require undue experimentation." (Office Action of 2 January 2002, pages 3-4). Applicants respectfully but emphatically disagree with this statement. Applicants believe that the scientific publications provided with the previous reponse make it abundantly clear that those of skill in the art do not consider these experiments to be undue. Rather, these sorts of experiments, with many variations and adaptations, are performed as a matter of course by those of skill in the art. Applicants have provided herewith a declaration of coinventor Heidi Major Sleister to show that one of skill in the art is able to perform the immunological aspects of the methods in a matter of days.

The Office Action disregards the teachings of the cited references and misrepresents the invention. The Office Action states that the art "only teaches the use of conformational antibody probes where the protein has an unmodified primary structure and where differences in the protein's conformation are due to its state of folding." (Office Action of 2 January 2002, page 3, #6). The Office Action continues, "[t]his is different from how conformational probes would be used in the instant invention, where changes are made in the amino acid sequence of a protein." Applicants agree that the methods of the instant invention differ from the prior art. However, as discussed above and in the Rule 132 declaration submitted herewith, the general immunological techniques used in the methods of the invention are readily performed by those of skill in the art.

The Examiner further questions whether "Applicants have been able to make changes to the primary structure of the disclosed VSPß and determine the proteins' conformation by the use of conformation-sensitive antibody probes." (Office Action of 2 January 2002, page 4, #6). Applicants herewith submit a Rule 132 declaration of coinventor Heidi Major Sleister to illustrate that such experiments, as described in the specification, may be readily carried out by those of skill in the art.

Applicants respectfully submit that for the reasons discussed above, the invention is fully enabled by the present specification and would not require undue experimentation. Accordingly,

the rejection under 35 U.S.C. §112, ¶ 1, should be withdrawn.

CONCLUSION

In view of the above amendments and remarks, Applicants submit that the rejection of the claims under 35 U.S.C. §112, first paragraph, is overcome. Applicants respectfully submit that this application is now in condition for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject Application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those, which may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

Leigh W. Thorne
Registration No. 47,992

CUSTOMER NO. 00826 **ALSTON & BIRD LLP** Bank of America Plaza 101 South Tryon Street, Suite 4000 Charlotte, NC 28280-4000 Tel Raleigh Office (919) 862-2200 Fax Raleigh Office (919) 862-2260	CERTIFICATE OF MAILING I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: **BOX AF**, Commissioner for Patents, Washington, DC 20231, on March 29, 2002. Lynda-Jo Pixley Lynda Jo Pixley

Version with Markings to Show Changes Made:

In the Specification:

Please revise the one-line paragraph beginning on page 3, line 2, to read as follows:

[Fig.] Figure 1 and Figure 1A show[s] VSP homologies.

In the Claims:

88. (Amended) The protein of Claim 87, wherein said essential amino acids are selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, [theronine] threonine and cysteine.

109. (Amended) The protein of Claim 108, wherein said essential amino acids are selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, [theronine] threonine and cysteine.

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PATENT

Attorney Docket No. 5718-16A (035718/193734)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Rao *et al.*	Confirmation No.:	1892
Appl. No.:	09/478,598	Group Art Unit:	1652
Filed:	January 6, 2000	Examiner:	P. Tung
For:	COMPOSITIONS AND METHODS FOR ALTERING AMINO ACID CONTENT OF PROTEINS		

March 25, 2002

Assistant Commissioner for Patents
Washington, DC 20231

RULE 132 DECLARATION
of
Heidi Major Sleister

Sir:

I, Heidi Major Sleister, Ph.D., do hereby declare and say as follows:

1. I am an inventor of the subject matter of the above-captioned application.

2. I am skilled in the art of the field of the invention. I have a Ph.D. in Biological Sciences from the University of Iowa. I have a Bachelor of Science degree in Biology from Central College. I have post-doctoral training from Dr. A. Gururaj Rao of Pioneer Hi-Bred in protein engineering. Since 1995, I have been engaged in the study of protein engineering. I have been employed by Pioneer Hi-Bred since 1995, and have been in their Traits and Technology Development Group since 1995.

3. I have read and understood the Office Actions in the above case dated July 16, 2001 and January 2, 2002.

4. Included with this declaration are copies of notebook pages from my laboratory notebook that I keep of my work at Pioneer Hi-Bred. The enclosed notebook pages describe experiments conducted by myself or Gururaj Rao, a supervisor in my laboratory of whose work I have firsthand knowledge.

5. These experiments are typical of immunologically-based experiments that would be performed in the practice of the invention. The methods of the invention involve altering the amino acid compositions of proteins of interest (*i.e.*, the protein's primary structure) while **substantially retaining the native conformation** of those proteins (*i.e.*, the protein's secondary and tertiary structure). To determine whether the native conformation of the protein has been retained, a molecule known to interact with the native conformation is used in a binding assay with the altered protein. Inability of a monoclonal antibody to bind the engineered protein of interest is an indication of changes in the conformation of the engineered protein of interest. Accordingly, the immunological methods involve the production and selection of a set of monoclonal antibodies which preferentially bind to the protein in its native conformation. As evidenced by the data presented in the attached laboratory notebook pages, each of these experiments can be performed within several days.

6. The creation and selection of a set of monoclonal antibodies which bind to the protein in its native conformation involves techniques which are commonly used in immunology. Thus, one of skill in the art can readily produce and identify a monoclonal antibody having the necessary properties for use in the methods of the invention. The attached laboratory notebook pages document the results of such experiments performed by me or the supervisor in my laboratory; I have firsthand knowledge of the work described in these experiments.

7. Monoclonal antibodies may be obtained commercially, from laboratories specializing in such services. Purified antigens are prepared and sent to a commercial laboratory where standard procedures are used to produce monoclonal antibodies in appropriate host animals. After several months, the commercial laboratory provides serum from the immunized animal. Antibody-producing cells from this serum are used to create pure cell lines producing monoclonal antibodies. Techniques to produce these cell lines are standard in the art. See, for example, Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), as cited on page 5 of the specification. These cell lines produce pure populations of monoclonal antibodies which are then used in the methods of the invention.

8. Experiment 1 employed a technique that can be described as a Competition ELISA. This experiment was designed to determine whether monoclonal antibodies that were raised against the VSP alpha and beta antigens bind to VSP antigens having the native conformation. The antigen was used to coat a 96-well microtiter plate. Separately, two aliquots of each monoclonal antibody were prepared. To one aliquot, buffer was added; to the other, a ten-fold molar excess of the antigen was added. These aliquots were preincubated for 15 minutes and then added to the microtiter plate that had been coated with antigen. A standard ELISA assay was then performed to detect antibody that bound to the microtiter plate.

In such an experiment, an antibody that recognizes native-conformation epitopes will bind to the antigen in solution in the preincubation step and thus will not be available to bind to the antigen on the microtiter plate. Conversely, an antibody that does not recognize native-conformation epitopes (*i.e.*, an antibody that recognizes denatured protein) will not bind to the antigen in solution in the preincubation step and thus will be available for binding to the antigen which is bound to the plate. (Note that antigen binds to a solid surface such as the microtiter plate in a somewhat random fashion, so that various epitopes are available for binding by bound antigen). Thus, this assay can be used to distinguish between antibodies which recognize the native conformation of the protein (*i.e.*, the conformation in solution) from antibodies which do not recognize the native conformation of the protein.

Here, the results indicated that all but one of the antibodies tested recognized the native conformation of the protein. This experiment illustrates that antibodies which detect the native conformation of the protein in solution may be readily identified using techniques known to those of skill in the art.

This experiment can be performed in as little as two days. For example, on the first day the microtiter plate would be coated with antigen and incubated overnight at 4°C. On the second day, the antibody to be tested would be preincubated with buffer or antigen for 15 minutes; the antibody aliquots would be added to the antigen-coated microtiter plate for an hour; the remainder of the standard ELISA steps would be performed in about 2 hours (including plate washing, incubation with antibody conjugate, and detection); and the evaluation of results would take about an hour.

9. Experiment 2 employed a technique that can be described as a Competition Protein A capture ELISA. This experiment was designed to determine whether the monoclonal antibodies recognize native or denatured epitopes on the antigen.

In this experiment, the antigen was labeled with biotin and then preincubated in solution with an antibody. This mixture was then added to a microtiter plate that had been coated with protein A. Protein A binds to antibodies, thus immobilizing the antibodies in the solution by binding them to the microtiter plate. Thus, the antibodies in the solution were immobilized on the microtiter plate. The amount of biotinylated antigen bound to the immobilized antibodies was then readily determined using streptavidin alkaline phosphatase with para-nitrophenylphosphate as a substrate.

In this experiment, competitors were be added to the preincubation solution to help determine the antibodies' binding characteristics. One of skill in the art is aware of modifications and adaptations that may be made to such experiments according to the question at hand. For example, here a constant amount of antibody plus biotinylated antigen in the preincubation solution was incubated with competitors comprising several dilutions of either native or heat-denatured, unlabelled antigen (see laboratory notebook page 21). If the antibody being tested binds to native epitopes on the antigen, then unlabeled native antigen will act as a competitor for binding to the antibody. The presence of unlabeled, bound antigen would be readily detected as a decrease in signal (*i.e.*, a reduction in the amount of absorbance detected in an ELISA). In contrast, if the antibody recognized a denatured epitope on the antigen, the addition of unlabelled, native-conformation antigen would not compete for binding to the antibody and thus the ELISA absorbance would not decrease.

Thus, this experiment was used to determine whether the antibody recognizes a native or denatured epitope on an antigen by determining which of these two competitors (native or denatured) acted as a competitor for the biotinylated antigen bound to the antibody. In Experiment 2 (see attached laboratory notebook pages), eleven monoclonal antibodies were identified as recognizing native, conformational epitopes on VSP. These antibodies may then be used in binding assays with VSP having altered amino acid content to determine whether the altered VSP retained the conformation of the native, unaltered protein.

This type of experiment can be performed in as little as three days. On days 1-2, the antigen would be biotinylated and the microtiter plate would be coated with protein A and incubated overnight at 4°C. On day 3, the biotinylated antigen, antibody, and unlabelled competitor antigen (either native or heat-denatured) would be preincubated for one hour and then added to the protein A-precoated microtiter plate and incubated for one hour. The plate would then be washed and the streptavidin alkaline phosphatase added and incubated for 30 minutes. The plate would then be washed again and substrate added (p-nitrophenylphosphate). The results of the assay are evaluated by comparing absorbance values.

10. Experiment 3 employed additive binding tests to determine whether two antibodies can bind an antigen simultaneously. This is helpful in determining whether two antibodies recognize the same epitope. Ideally, a panel of antibodies recognizing different conformational epitopes are used to evaluate the conformation of the altered protein of interest. The use of a range of antibodies helps assure that the conformation of the altered protein has not been changed.

In this experiment, saturating amounts of antibodies were incubated with the antigen as follows. A microtiter plate was coated with a low concentration of the antigen. Monoclonal antibodies were then tested in pairs. A saturating concentration of each antibody was added to separate antigen-coated wells, and saturating amounts of pairs of antibodies were combined and added to a single antigen-coated well. After incubation, the entire microtiter plate was washed and an immunoconjugate was added to detect the amount of antibody bound to the antigen. If the two antibodies in a pair recognize the same epitope, the quantitative ELISA result for this pair should be equal to the average of the result from the antibodies tested separately. In contrast, if the antibodies in a pair recognize different epitopes on the same antigen, then the ELISA result (*i.e.*, the absorbance) of the two antibodies together should be greater than the ELISA absorbance of either antibody tested alone. In fact, in this case the absorbance should approximate the sum of the absorbance values derived from each of the two antibodies tested separately.

In Experiment 3 (see attached notebook pages), eleven monoclonal antibodies were screened for their ability to simultaneously bind the same antigen. The results indicate that most of the antibodies screened can bind to their epitope at the same

time that another antibody is bound. Consequently, these antibodies recognize different epitopes of VSP and therefore would be useful together in ascertaining whether a VSP with altered amino acid composition had retained the native conformation of VSP.

This type of experiment can be performed in three days. On days 1 and 2, the person performing the test determines the amount of each antibody required to saturate a given amount of antigen. See, for example, Friguet (1989) "Immunochemical analysis of protein conformation," in *Protein structure: a practical approach*, ed. Creighton (IRL Press at Oxford University Press, Oxford) (previously submitted and discussed in Applicants' Response of October 16, 2001). A microtiter plate is coated with a small amount of antigen and incubated overnight at 4°C. On day 3, saturating amounts of each antibody are added alone and in pairs to the antigen on the microtiter plate; this is incubated for an hour. The remainder of the ELISA is then performed, including plate washing, incubation with antibody conjugate, and detection; these steps take about two hours. Results are then evaluated by comparing the absorbance values between the antibodies incubated with antigen individually with the results from incubations of pairs of antibodies. These values may be compared with the use of an "additivity index" as described in the Friguet reference, *supra* at page 298. The analysis takes about two hours.

11. Experiment 4 illustrates another technique that can be used to determine whether VSP-specific antibodies recognize native or denatured antigen. VSP protein was run on an SDS-polyacrylamide denaturing gel, which denatures proteins. The resulting gel was transferred to a nylon membrane for the remainder of the Western blot analysis with VSP-specific antibodies. Binding of antibodies was detected using anti-mouse IgG-biotin conjugate, ExtrAvidin[1]- alkaline phosphatase, and substrate BCIP/NBT. The results (see attached laboratory notebook pages) show that only one of ten monoclonal antibodies reacted with the denatured VSP on the blot. Thus, most of these monoclonal antibodies do not react with denatured VSP.

12. Experiment 5 was conducted to evaluate VSPß-Met10, a protein engineered for increased methionine content and described in the specification, particularly on page 19 and in Table 2. This VSPß variant and the control wildtype VSPß protein (VSPß-WT) were evaluated using VSP-specific antibodies that recognize native,

[1] ExtrAvidin is a modified streptavidin commercially available from Sigma Chemical Co.

conformational VSP epitopes. On day 1, equal amounts of either VSPß-WT or VSPß-Met10 were immobilized to separate microtiter wells and incubated overnight at 4°C. On day 2, VSP-specific monoclonal antibodies were added to the microtiter wells and incubated for an hour at 37°C. The remainder of the ELISA was then performed, including washing the plate, adding anti-mouse IgG-biotin conjugate and streptavidin alkaline phosphatase, and incubating with substrate p-nitrophenylphosphate.

If these antibodies had not recognized VSP in this ELISA, the absorbance values would have been equal to the negative, background-level control. Thus, the results of this experiment (see attached laboratory notebook pages) indicate that nearly all of the conformational antibodies recognize refolded VSPß-Met10, leading to the conclusion that this methionine-enriched VSPß variant is correctly folded.

13. Thus, the experiments performed above illustrate the types of tests that are useful in the practice of the invention. As shown by the results and notebook records of these experiments, one of skill in the art can readily perform such a series of experiments with a reasonable amount of effort in a reasonable amount of time.

14. For the above reasons and based on my education and scientific experience, I believe that the claims drawn to methods for altering the amino acid compositions of proteins of interest while substantially retaining the native conformation of those proteins are fully enabled and described by the specification. I further believe that the amount of experimentation needed to perform the methods of the claims is readily achieved and is not an unusual or undue amount of experimentation.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 3/27/02 By: Heidi Major Sleister

Heidi Major Sleister

EXPERIMENT 1

Appl. No. 09/478,598
Filed: January 6, 2000

DISCRIMINATION BETWEEN ANTI-NATIVE & ANTI-DENATURED ANTIBODIES

Antibody solution (2 ml)

2 ug/ml (0.002 mg/ml) in PBS-Tween = 13.33 nanomolar

Antigen solution (2 ml)

10-fold higher over antibody concentration: 133 nanomolar or 0.133 uM in PBS-Tween

2 ml Antibody

1 ml antibody + 1 ml buffer | 1 ml antibody + 1 ml antigen

Incubate for 15 min at room temperature

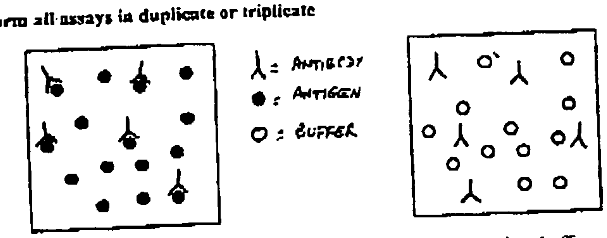
Add 200 ul to antigen-coated well | Add 200 ul to antigen-coated well

Incubate for 60 min at room temperature

Rinse 3 times with PBS-tween | Rinse 3 times with PBS-tween

Proceed with ELISA as before

Note: Perform all assays in duplicate or triplicate



Antibody + excess Antigen (Figure 1)

Antibody + buffer (Figure 2)

Interpretation

(1) If the antibody tested is anti-native (Figure 1), there will not be any free antibody to interact with the antigen coated on the plate. Consequently, the absorbance will be much lower than obtained in wells incubated with antibody alone (Figure 2).

(2) If the antibody tested does not recognize native epitopes, there is enough antibody to recognize antigen coated on the plate. Consequently, the abosrbance measured will be similar to that obtained in wells incubated with antibody alone.

Some

Witnessed

Date

Date

[illegible] at 405 nm, 80 min after addition of substrate

	BEFORE SUBTRACTING BLANK			AFTER SUBTRACTING BLANK		
	Antibody + buffer	Antibody + VSP	Blank Avg = 0.059	Antibody + buffer	Antibody + VSP	
[illegible] 11	0.166, 0.162	0.074, 0.070	0.073	0.107, 0.103.	0.015, 0.011	N
[illegible] 12	0.258, 0.251	0.199, 0.199	0.051	0.207, 0.200	0.148, 0.148	D?
[illegible] 10	0.281, 0.271	0.094, 0.095	0.054	0.222, 0.212	0.035, 0.036	N
[illegible] B3	0.276, 0.273	0.107, 0.109	0.050	0.217, 0.214	0.048, 0.050	N
[illegible] E3	0.471, 0.479	0.201, 0.191	0.063	0.412, 0.420	0.142, 0.132	N
[illegible] D6	0.261, 0.263	0.075, 0.078	0.056.	0.202, 0.204	0.016, 0.019	N
[illegible] D5	0.290, 0.297	0.096, 0.100	0.072.	0.231, 0.238	0.037, 0.041	N
VII C 10	0.455, 0.459	0.241, 0.230	0.054	0.396, 0.[illegible]	0.182, 0.171	N
II E 6	0.476, 0.460	0.227, 0.222	0.073	0.417, 0.401	0.168, 0.163.	N
VII B 9	0.225, 0.268	0.056, 0.057.	0.051	0.166, 0.209	0.03, 0.03,	N
IX D 5	0.097, 0.103	0.058, 0.053	0.054	0.038, 0.044	0.001, 0.005	N
V C 5	0.273, 0.270	0.080, 0.082	0.050	0.214, 0.211	0.021, 0.023	N
V F 5	0.136, 0.166	0.064, 0.085	0.054.	0.077, 0.107	0.005, 0.026.	N
I G 7	0.279, 0.288	0.067, 0.069	0.063	0.220, 0.229	0.008, 0.01.	N

Witnessed [signature] [signature]

Date

EXPERIMENT 2

Appl. No. 09/478,598
Filed: January 6, 2000

21 Competition PACE - Testing ability of denatured VSP to bind "conformational" mAbs

Date

Purpose: To determine whether VSP-specific mAbs are conformational (i.e. - recognize native epitopes)

Method: Using PACE - inc. of mAbs + (b)VSP + VSP competitor. Competitor is either native VSP or denatured VSP. Here, denaturation will be achieved by heat (65C, 15') or exposure to guanidine thiocyanate (GTC).

Expected results:

(b)VSP	mAb	competitor	O.D. value
(b)VSP (20nM)	2nM	-	1X
(b)VSP (20nM)	2nM	20nM native VSP	1/2 X
(b)VSP (20nM)	2nM	4nM native VSP	1/2 X < (?) < X
(b)VSP (20nM)	2nM	20nM denatured VSP	X (if completely denatured & mAb recognizes native ep.)
"	"	"	1/2 X if completely denatured & mAb recognizes denatured epitope

· If competitor is partially denatured, I'd expect intermediate values.

Making stocks of reagents (10X) to use in competition PACE -

(b)VSP is at 2.9μM → made 1mL 200nM stock (69μL 2.9μM (b)VSP + 931 PBS)
unbiotinylated VSP is @ .153mg/mL or ... " "
" " " → made 500μL 40nM stock (6.9μL VSP ... + 493.1μL PBS)

Made 20nM stocks of all mAbs (from PFM - stored @ 4°C)

Dilutions to make 20nM stocks - made 200μL - values for 100μL below

mAb	conc stock (IgG μM)	μL mAb	μL PBS	mAb	conc stock	μL mAb	μL PBS
1B6	2.6 μM	.77	99.2	4F6	2.17 μM	.92	99.1
1G5	.073 μM	27.4	72.6	4E12	3.34 μM	.60	99.4
1G7	3.9 μM	.51	99.5	5C5	1.85 μM	1.08	99
2E4	.147 μM	13.6	86.4	6F12	1.88 μM	1.06	99
3B3	3.91 μM	.51	99.5	7C10	2.87 μM	.7	99.3
3E3	.64 μM	3.13	96.9	9D5	.32 μM	6.25	93.8
4E12	8.47 μM	.24	99.75				

Signed Heidi Sleister

Date

Witnessed Teresa Thompson

Date

Date

Row	(b) VSP	mAb	competitor
A-	20 nM	2 nM	0
B-			20 nM native soybean VSP
C-			4 nM "
D-			20 nM GTC-denatured VSP*
E-			4 nM "
F-			20 nM heat (65°C, 15') denatured VSP
G-			4 nM "
H-	0		0

denaturation by GTC:
6.7 µl 6M GTC +
① 0.3 µl VSP (@ 6.85 mg/ml) + 3 λ H_2O
② 2.8 µl VSP (@ .153 mg/ml) + 0.5 λ H_2O
~~4000~~ 21.5 hrs, RT.

Added 190 µl PBS - GTC conc. is now 0.2 M. This becomes 0.02 M when this GTC-denatured VSP is added to the preincubation mixture.

*① above is for 200 nM VSP-GTC "stock"
*② above is for 40 nM VSP-GTC "stock."

Preincubated: added 11 µl (b) VSP, 11 µl mAb, 11 µl (or zero for row A) competitor + enough PBS-BSA to give 110 µl. Added this mixture to a preblocked microtiter well. Below is a list of mAbs added. (duplicates done)

Plate #	column #	mAb
1	2, 3	1B6
1	4, 5	1G5
1	6, 7	1G7
1	8, 9	2E6
1	10, 11	3B3
2	2, 3	3E3
2	4, 5	4E12
2	6, 7	4F10
2	8, 9	4F12
2	10, 11	5C5
3	2, 3	6F12
3	4, 5	7C10
3	6, 7	9D5

(results on following pages.) →

Preinc. 1 hr, then transferred (50 µl per well) to Protein A-coated wells. 1.5 hr, 37C. Washed 3X PBST. Added 100 µl extravidin (1:50K dil in PBST). ~~Washed 3~~ 1 hr, 37C. Washed 3X PBST. Added 100 µl 2 mg/ml PNPP.

Signed Heidi Sleister

Date

Witnessed Teresa Thompson

Date

- Cont'd - Results - Plate 1

Date

mAb

Pt1 39 min	1	1B6 2	1B6 3	1G5 4	1G5 5	1G7 ~~Plate#4~~ 6	1G7 7	2E6 8	2E6 9	3B3 10	3B3 11	Competitor ↓
A	0.106	0.235	0.177	0.109	0.117	0.146	0.148	0.308	0.314	0.162	0.149	0 (pos. control)
B	0.103	0.142	0.123	0.103	0.100	0.110	0.114	0.138	0.133	0.113	0.114	~~20nM native USP~~
C	0.099	0.172	0.165	0.106	0.104	0.126	0.126	0.198	0.206	0.140	0.144	4nM "
D	0.095	0.160	0.145	0.107	0.103	0.126	0.132	0.233	0.246	0.149	0.144	20nM } GTC + denatured USP
E	0.098	0.163	0.170	0.104	0.105	0.140	0.145	0.247	0.267	0.148	0.154	4nM }
F	0.097	0.138	0.143	0.099	0.102	0.111	0.111	0.147	0.162	0.122	0.123	20nM } 65°C + denatured USP
G	0.096	0.172	0.184	0.109	0.123	0.119	0.122	0.235	0.248	0.144	0.149	4nM }
H	0.102	0.110	0.099	0.098	0.099	0.097	0.099	0.100	0.106	0.099	0.107	← ~~neg control~~

39 min

Pt1 148 min	1	2	3	4	5	6	7	8	9	10	11
A	0.117	0.511	0.327	0.134	0.152	0.235	0.235	0.717	0.722	0.283	0.230
B	0.116	0.211	0.181	0.117	0.115	0.133	0.142	0.229	0.206	0.147	0.139
C	0.114	0.326	0.304	0.127	0.120	0.184	0.186	0.394	0.417	0.225	0.245
D	0.102	0.285	0.246	0.130	0.119	0.189	0.206	0.497	0.532	0.257	0.239
E	0.103	0.299	0.318	0.123	0.128	0.235	0.250	0.534	0.596	0.252	0.269
F	0.104	0.227	0.240	0.111	0.120	0.148	0.149	0.247	0.283	0.183	0.182
G	0.103	0.330	0.362	0.127	0.137	0.171	0.182	0.504	0.536	0.246	0.251
H	0.113	0.119	0.106	0.104	0.108	0.104	0.106	0.110	0.116	0.106	0.107

148 min

Pt1 20hrs 34 min	1	2	3	4	5	6	7	8	9	10	11
A	0.220	2.433	1.422	0.308	0.411	0.910	0.877	3.310	3.347	1.191	0.886
B	0.205	0.729	0.609	0.226	0.224	0.310	0.327	0.854	0.744	0.402	0.309
C	0.168	1.458	1.328	0.284	0.250	0.639	0.640	1.854	1.982	0.869	0.935
D	0.148	1.177	0.996	0.311	0.239	0.651	0.749	2.403	2.588	1.055	0.962
E	0.145	1.302	1.428	0.260	0.300	0.932	1.022	2.662	3.023	1.038	1.130
F	0.154	0.896	0.959	0.204	0.248	0.420	0.429	1.005	1.197	0.630	0.627
G	0.157	1.467	1.685	0.284	0.304	0.570	0.614	2.518	2.685	1.014	1.018
H	0.180	0.163	0.158	0.153	0.171	0.157	0.153	0.187	0.185	0.159	0.162

20 hrs 34 min

Signed Heidi Sleisser

Date

Witnessed Teresa Thompson

Date

Contd - Results - Plate 2

Date

plt 2 21.5 min	1	2 (mAb 3E3)	3 (mAb 3E3)	4 (mAb 4E12)	5 (mAb 4E12)	6 (mAb 4F10 ~~Plate#1~~)	7 (mAb 4F10 ~~Plate#1~~)	8 (mAb 4F12)	9 (mAb 4F12)	10 (mAb 5C5)	11 (mAb 5C5)	competitor ↓
A	0.110	0.143	0.168	0.153	0.179	0.933	0.957	0.359	0.381	0.151	0.118	0 (pos. control)
B	0.099	0.108	0.119	0.106	0.113	0.362	0.377	0.121	0.117	0.112	0.113	20 nM } native VSP
C	0.098	0.119	0.116	0.126	0.135	0.679	0.680	0.197	0.197	0.143	0.149	4 nM
D	0.098	0.125	0.121	0.113	0.123	0.707	0.695	0.226	0.223	0.159	0.156	20 nM } ETC-denatured VSP
E	0.102	0.124	0.123	0.116	0.130	0.738	0.768	0.257	0.257	0.177	0.186	4 nM
F	0.096	0.106	0.106	0.111	0.123	0.472	0.478	0.133	0.137	0.146	0.150	20 nM } GSC-denatured VSP
G	0.098	0.123	0.141	0.138	0.148	0.777	0.811	0.265	0.261	0.192	0.195	4 nM
H	0.100	0.098	0.105	0.100	0.100	0.100	0.100	0.101	0.101	0.100	0.101	neg control

plt 2 9 min	1	2 (3E3)	3 (3E3)	4 (4E12)	5 (4E12)	6 (4F10 Plate#1)	7 (4F10 Plate#1)	8 (4F12)	9 (4F12)	10 (5C5)	11 (5C5)
A	0.109	0.179	0.190	0.214	0.259	1.640	1.708	0.607	0.700	0.216	0.144
B	0.104	0.121	0.131	0.117	0.125	0.603	0.631	0.146	0.138	0.130	0.132
C	0.101	0.141	0.134	0.152	0.171	1.164	1.184	0.292	0.297	0.194	0.204
D	0.104	0.154	0.147	0.133	0.155	1.210	1.198	0.354	0.348	0.223	0.224
E	0.106	0.154	0.152	0.141	0.163	1.290	1.364	0.409	0.416	0.258	0.284
F	0.100	0.120	0.193	0.130	0.144	0.822	0.837	0.173	0.181	0.203	0.213
G	0.102	0.152	0.162	0.172	0.203	1.346	1.453	0.419	0.420	0.287	0.296
H	0.102	0.102	0.121	0.105	0.104	0.104	0.105	0.107	0.106	0.104	0.105

	1	2	3	4	5	6	7	8	9	10	11
A	0.142	0.378	0.367	0.483	0.617	3.782	4.000	1.694	1.762	0.478	0.252
B	0.125	0.180	0.188	0.166	0.190	1.704	1.768	0.257	0.231	0.203	0.211
C	0.117	0.246	0.221	0.281	0.341	3.796	4.000	0.720	0.727	0.401	0.433
D	0.125	0.289	0.271	0.219	0.277	3.388	3.313	0.914	0.894	0.495	0.505
E	0.125	0.297	0.293	0.244	0.322	4.000	4.000	1.074	1.110	0.604	0.683
F	0.115	0.184	0.227	0.212	0.261	2.423	2.460	0.348	0.378	0.437	0.467
G	0.120	0.282	0.284	0.344	0.450	4.000	4.000	1.126	1.107	0.693	0.724
H	0.118	0.114	0.128	0.120	0.131	0.121	0.124	0.126	0.127	0.121	0.122

Signed Heidi Sneisser Date Witnessed [signature] Date

3 contd   Results - Plate 3

Date

P143 4 min	1	2 (In Pos GF12)	3 (In Pos GF12)	4 (7cic)	5 (7cic)	6 (9D5)	7 (9D5)	Competitor
A	0.116	0.415	0.367	0.264	0.254	0.225	0.220	0 (pos. control)
B	0.115	0.149	0.175	0.139	0.141	0.191	0.199	20 nM native VSP
C	0.110	0.273	0.224	0.181	0.189	0.180	0.211	4 nM native VSP
D	0.104	0.256	0.244	0.214	0.213	0.179	0.182	20 nM GTC-denatured VSP
E	0.094	0.264	0.254	0.214	0.216	0.172	0.172	4 nM GTC-denatured VSP
F	0.094	0.167	0.179	0.160	0.162	0.174	0.176	20 nM 65C-denatured VSP
G	0.088	0.228	0.235	0.197	0.218	0.188	0.182	4 nM 65C-denatured VSP
H	0.102	0.177	0.103	0.109	0.106	0.109	0.112	neg control

Plate#1

56 min

Pl 3 - 100'

Plate#1

	1	2	3	4	5	6	7
A	0.146	0.702	0.604	0.414	0.401	0.338	0.327
B	0.133	0.196	0.230	0.180	0.184	0.288	0.300
C	0.121	0.417	0.330	0.272	0.276	0.268	0.276
D	0.112	0.428	0.382	0.331	0.355	0.267	0.256
E	0.104	0.423	0.425	0.330	0.340	0.256	0.237
F	0.108	0.242	0.267	0.227	0.232	0.254	0.259
G	0.097	0.347	0.363	0.303	0.316	0.285	0.263
H	0.113	0.148	0.112	0.120	0.115	0.122	0.127

100 min

See comments next page

P143 168 min

Plate#1

	1	2	3	4	5	6	7
A	0.153	1.120	0.967	0.623	0.585	0.498	0.492
B	0.153	0.269	0.299	0.229	0.234	0.398	0.422
C	0.135	0.599	0.490	0.362	0.379	0.364	0.432
D	0.123	0.625	0.548	0.471	0.481	0.363	0.369
E	0.115	0.632	0.602	0.475	0.472	0.341	0.343
F	0.116	0.326	0.360	0.312	0.304	0.353	0.354
G	0.108	0.533	0.535	0.425	0.450	0.380	0.380
H	0.131	0.164	0.125	0.132	0.130	0.141	0.145

168 min

Signed Heidi Steiger   Date

Witnessed Teresa Thompson   Date

cont'd

Comments -

It appears as if the O.D.'s w/ denatured VSP are higher (rows D+E) than w/ native VSP (rows B, C) used as a competitor. However, the O.D.'s w/ GTC-VSP are lower than w/o a competitor, suggesting that either ① there's a small population of renatured / undenatured VSP in the GTC or heat-treated VSP sample, or ② the small amt of remaining GTC (.02M) is sufficient to denature some of the other proteins in the assay (eg. - protein A, mAb) such that the O.D. is reduced.

To determine whether #2's correct, did experiment described on next pg.

Signed Heidi Sleister

Date

Witnessed Teresa Thompson

Date

PACE - VSP-specific mAbs w/ biotinylated VSP + native VSP + denatured VSP competitors   Date

Purpose: repeating previous exp. in triplicate + buffer controls

Methods: As described previously - loadings into microtiter wells as follows -

Row	(200nM) biotin-VSP	(20nM) mAbs	competitor	BSA	PBS	total
A	17	17	0	119	17	170
B			17µl native VSP (200nM)		0	
C			* 17λ buffer w/ GTC		0	
D			17λ GTC-denatured VSP (200nM)		0	
E			* 17λ buffer (heated to 65C)		0	
F			17λ heat-denatured VSP (200nM)		0	
G	0		0		34	

* Controls of buffer alone (GTC or heated)

Amts. listed above are for preincubations. After preinc (1hr on rocker @RT), added 50µl to Protein A coated well → in triplicates. Inc 1hr, 37C. Washed 3X PBST. Added 100µl 1:50K extravidin in PBST. Inc 50min 37C. Washed 3X PBST. Added 100µl 2mg/ml PNPP in diethanolamine buffer. Read O.D.'s over ~2hr time period @ 405nm.

Antibodies used:

Plate - 1	Columns	1,2,3	mAb - 1B6	See results on following pages
1		4,5,6	1G7	
1		7,8,9	2E6	
1		10,11,12	3B3	
Plt. 2	Columns	1,2,3	3E3	
		4,5,6	4E12	
		7,8,9	4F12	
		10,11,12	5C5	
Plt. 3	Columns	2,3,4	6F12	
		5,6,7	7C10	
		8,9,10	9D5	

Denaturation w/ GTC was done as described on p. 22. Heat denaturation was 65C for 25 mins, then iced immediately & briefly spin down tube

Signed Heidi Sneisav   Date   Witnessed Teresa Thompson   Date

31 Plate 1 results

Date

Plate#1

	1B6			1G7			2E6			3B3			
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.243	0.231	0.234	0.159	0.221	0.249	0.488	0.520	0.573	0.217	0.219	0.230	17 min
B	0.152	0.147	0.152	0.107	0.120	0.133	0.214	0.239	0.427	0.184	0.119	0.122	
C	0.243	0.237	0.230	0.118	0.179	0.290	0.387	0.458	0.633	0.200	0.208	0.219	
D	0.219	0.211	0.218	0.120	0.165	0.229	0.428	0.469	0.504	0.176	0.182	0.179	
E	0.203	0.196	0.201	0.143	0.147	0.148	0.385	0.495	0.525	0.166	0.167	0.170	
F	0.208	0.203	0.206	0.106	0.133	0.247	0.450	0.488	0.500	0.176	0.164	0.184	
G	0.096	0.097	0.096	0.095	0.096	0.096	0.100	0.102	0.102	0.095	0.095	0.097	

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.721	0.682	0.691	0.374	0.643	0.754	1.740	1.824	2.019	0.578	0.596	0.633	82 min
B	0.339	0.326	0.336	0.144	0.200	0.249	0.579	0.667	1.444	0.477	0.184	0.207	
C	0.721	0.695	0.662	0.210	0.443	0.901	1.252	1.508	2.162	0.505	0.536	0.562	
D	0.627	0.585	0.614	0.194	0.387	0.643	1.367	1.500	1.607	0.407	0.409	0.412	
E	0.562	0.528	0.555	0.300	0.317	0.317	1.226	1.598	1.667	0.363	0.366	0.380	
F	0.591	0.561	0.572	0.147	0.260	0.732	1.519	1.625	1.573	0.389	0.339	0.406	
G	0.101	0.100	0.098	0.097	0.098	0.101	0.114	0.122	0.146	0.097	0.096	0.101	

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.166	0.162	0.163	0.712	0.683	0.687	1.964	1.984	1.923	0.352	0.352	0.376	147 min
B	0.134	0.125	0.125	0.302	0.309	0.319	1.095	1.113	1.106	0.155	0.154	0.159	
C	0.164	0.153	0.151	0.539	0.654	0.546	1.742	1.778	1.802	0.293	0.302	0.307	
D	0.147	0.143	0.138	0.426	0.413	0.412	1.553	1.478	1.562	0.220	0.239	0.236	
E	0.157	0.145	0.146	0.560	0.580	0.585	1.487	1.464	1.535	0.207	0.205	0.211	
F	0.148	0.138	0.142	0.499	0.509	0.512	1.667	1.688	1.649	0.276	0.281	0.286	
G	0.101	0.100	0.099	0.097	0.098	0.103	0.098	0.099	0.100	0.097	0.103	0.098	

* Exp done

data entered

Signed Heidi Sleister Date

Witnessed Teresa Thompson Date

contd Plate 2 results

Date

		3E3			4E12			Plate#1 4F12			SC5			
		1	2	3	4	5	6	7	8	9	10	11	12	
Hbno	A	0.115	0.114	0.123	0.278	0.270	0.282	0.708	0.793	0.746	0.189	0.195	0.201	9 min
	B	0.107	0.105	0.104	0.163	0.159	0.162	0.407	0.428	0.448	0.117	0.116	0.124	
	C	0.112	0.111	0.111	0.217	0.234	0.231	0.589	0.620	0.613	0.165	0.170	0.183	
	D	0.107	0.109	0.108	0.187	0.190	0.185	0.504	0.490	0.569	0.140	0.152	0.146	
	E	0.111	0.108	0.109	0.205	0.216	0.225	0.481	0.464	0.509	0.133	0.130	0.132	
	F	0.107	0.107	0.111	0.213	0.206	0.210	0.553	0.539	0.511	0.150	0.151	0.158	
	G	0.097	0.097	0.097	0.095	0.095	0.098	0.096	0.099	0.098	0.096	0.097	0.096	
Hbno	A	0.145	0.140	0.141	0.501	0.492	0.507	1.371	1.417	1.371	0.284	0.278	0.296	32 min
	B	0.124	0.116	0.115	0.230	0.237	0.248	0.783	0.767	0.752	0.137	0.134	0.144	
	C	0.143	0.134	0.132	0.387	0.401	0.398	1.213	1.205	1.224	0.228	0.236	0.246	
	D	0.130	0.127	0.124	0.315	0.312	0.308	1.072	1.010	1.055	0.172	0.189	0.193	
	E	0.137	0.128	0.129	0.410	0.414	0.419	1.019	0.991	1.018	0.167	0.163	0.169	
	F	0.131	0.124	0.131	0.356	0.358	0.366	1.144	1.128	1.100	0.208	0.211	0.220	
	G	0.099	0.099	0.098	0.095	0.097	0.101	0.096	0.114	0.098	0.096	0.102	0.096	
Hbno	A	0.218	0.215	0.218	1.204	1.173	1.189	4.000	4.000	3.612	0.544	0.537	0.569	80 min
	B	0.159	0.146	0.147	0.504	0.485	0.507	1.974	1.980	1.843	0.201	0.195	0.202	
	C	0.219	0.199	0.197	0.909	0.935	0.946	3.428	3.563	3.306	0.442	0.458	0.457	
	D	0.195	0.180	0.175	0.703	0.687	0.686	2.799	2.678	2.581	0.305	0.333	0.328	
	E	0.201	0.185	0.186	0.960	0.995	1.020	2.792	2.689	2.671	0.284	0.280	0.294	
	F	0.189	0.173	0.176	0.836	0.862	0.872	3.910	3.653	3.111	0.399	0.404	0.415	
	G	0.108	0.106	0.104	0.101	0.102	0.103	0.100	0.101	0.102	0.098	0.098	0.100	
Hbno	A	0.273	0.266	0.267	1.611	1.535	1.550	4.000	4.000	4.000	0.735	0.730	0.764	120 min
	B	0.185	0.168	0.170	0.620	0.609	0.633	2.495	2.514	2.477	0.244	0.238	0.250	
	C	0.279	0.242	0.238	1.190	1.211	1.201	4.000	4.000	4.000	0.572	0.597	0.613	
	D	0.246	0.217	0.217	0.915	0.887	0.892	3.432	3.275	3.333	0.388	0.420	0.440	
	E	0.249	0.223	0.223	1.243	1.285	1.294	3.818	3.614	3.866	0.356	0.355	0.373	
	F	0.232	0.207	0.210	1.110	1.131	1.137	4.000	4.000	4.000	0.516	0.530	0.546	
	G	0.115	0.111	0.109	0.105	0.106	0.107	0.103	0.118	0.105	0.102	0.100	0.103	

Heidi Sleister

Teresa Thompson

33 contd Plate 3 Results

Date

	1	2 (6F12)	3 (6F12)	4 (6F12)	5 (7C10)	6 (7C10)	7 (7C10)	8 (9D5)	9 (9D5)	10 (9D5)
					Plate#1					
1 hrs A	0.058	0.556	0.554	0.563	0.341	0.348	0.349	0.347	0.349	0.345
B	0.054	0.215	0.217	0.218	0.178	0.185	0.184	0.319	0.322	0.342
C	0.055	0.500	0.498	0.492	0.342	0.347	0.356	0.339	0.348	0.349
D	0.054	0.437	0.437	0.439	0.284	0.283	0.294	0.338	0.343	0.360
E	0.054	0.368	0.379	0.374	0.298	0.304	0.308	0.474	0.490	0.476
F	0.054	0.415	0.420	0.414	0.273	0.287	0.288	0.344	0.340	0.342
G	0.054	0.100	0.099	0.098	0.096	0.095	0.096	0.096	0.096	0.099
H	0.069	0.111	~~0.054~~	~~0.052~~	0.054	~~0.054~~	~~0.060~~	~~0.054~~	~~0.063~~	~~0.058~~
2 hrs A	0.058	0.758	0.746	0.763	0.461	0.462	0.462	0.462	0.461	0.440
B	0.054	0.277	0.273	0.271	0.216	0.222	0.220	0.409	0.412	0.426
C	0.056	0.709	0.663	0.678	0.435	0.681	0.452	0.441	0.442	0.442
D	0.056	0.584	0.595	0.587	0.362	0.355	0.360	0.437	0.426	0.450
E	0.054	0.497	0.505	0.487	0.381	0.381	0.383	0.611	0.610	0.588
F	0.055	0.570	0.559	0.554	0.348	0.363	0.363	0.440	0.419	0.412
G	0.055	0.102	0.107	0.100	0.097	0.097	0.097	0.097	0.097	0.100
H	0.068	~~0.113~~	~~0.056~~	~~0.054~~	~~0.055~~	~~0.054~~	~~0.061~~	~~0.055~~	~~0.065~~	0.062

There are additional time pts that were taken, but here I've shown ones that give good data — in the range of O.D.s ~0.3 → 0.8.

Signed Heidi Sleister Date

Witnessed Teresa Thompson Date

cont'd Spreadsheet of data used to make histogram on next pg.

Date

The data here is in the range of 0.3-0.8 for the positive control (row A data). This data was used to make the histogram on the next pg.

corrected = mean - blank

HHS

time pt	mAb	blank	rdg. At 405 nm w/o compet			mean	corrected	blank	w/heated VSP compet			mean	corrected	reduction	% reduction
62 MIN	1B6	0.100	0.721	0.662	0.691	[illegible]	0.595	0.100	0.339	0.326	0.336	[illegible]	0.234	0.608	60.825
47 min	1G7	0.102	0.712	0.683	0.687	[illegible]	0.592	0.099	0.302	0.309	0.318	[illegible]	0.211	0.644	64.368
17 min	2E6	0.099	.485	0.520	0.673	[illegible]	0.426	0.099	0.214	0.239	0.427	[illegible]	0.194	0.546	54.385
62 MIN	3B3	0.098	0.578	0.590	0.633	[illegible]	0.504	0.098	0.477	0.164	0.207	[illegible]	0.191	0.621	62.082
120 MIN	3E3	0.112	0.273	0.269	0.267	[illegible]	0.157	0.112	0.185	0.168	0.170	0.174	0.062	0.602	60.213
62 MIN	4E12	0.098	0.501	0.492	0.507	[illegible]	0.402	0.098	0.230	0.227	0.248	[illegible]	0.140	0.651	65.091
9 MIN	4F12	0.096	0.706	0.793	0.748	[illegible]	0.651	0.096	0.407	0.426	0.446	[illegible]	0.330	0.494	49.350
60 MIN	5C5	0.099	0.544	0.537	0.569	[illegible]	0.451	0.099	0.201	0.195	0.202	[illegible]	0.100	0.778	77.753
44 MIN	6F12	0.099	0.556	0.954	0.589	[illegible]	0.436	0.099	0.215	0.217	0.218	[illegible]	0.116	0.743	74.346
62 MIN	7C10	0.097	0.461	0.462	0.462	0.462	0.365	0.097	0.216	0.222	0.220	[illegible]	0.122	0.665	66.453
62 MIN	9D5	0.096	0.482	0.461	0.440	0.454	0.358	0.096	0.409	0.412	0.425	0.416	0.318	0.109	10.861

reduction = mean O.D. w/ native competitor ÷ mean O.D. w/o competitor

$\times 100 = \uparrow$

HMO

mAb	control	w/GTC/VSP compet			mean	mean/control	%reduction	control	w/heated VSP compet			mean	mean/control	%reduction	% red
1B6	0.650	0.627	0.565	0.614	0.609	0.878	12.169	0.548	0.591	0.561	0.572	0.575	1.049	-4.968	.1[illegible]
1E7	0.548	0.426	0.413	0.412	0.417	0.764	23.620	0.675	0.499	0.509	0.512	0.507	0.861	11.664	.241
2E6	0.493	0.426	0.409	0.500	0.456	0.915	8.644	0.460	0.430	0.456	0.500	0.479	1.024	-2.422	-.0[illegible]
3B3	0.534	0.407	0.409	0.412	0.409	0.767	23.346	0.256	0.363	0.365	0.380	0.370	1.444	-44.401	.335
3E3	0.253	0.246	0.217	0.217	0.227	0.896	10.408	0.231	0.232	0.207	0.210	0.216	0.937	6.349	.117
4E12	0.395	0.315	0.312	0.300	0.312	0.789	21.097	0.414	0.356	0.358	0.366	0.360	0.870	13.043	[illegible]
4F12	0.607	0.504	0.490	0.569	0.521	0.858	14.169	0.485	0.653	0.539	0.511	0.534	1.102	-10.172	.2[illegible]
5C5	0.452	0.306	0.333	0.326	0.322	0.712	20.761	0.286	0.399	0.404	0.416	0.406	1.420	-41.958	.2[illegible]
6F12	0.496	0.437	0.437	0.439	0.438	0.882	11.761	0.374	0.415	0.420	0.414	0.416	1.113	-11.319	.29
7C10	0.522	0.362	0.355	0.360	0.359	0.688	31.226	0.382	0.346	0.363	0.363	0.358	0.937	6.283	.225
9D5	0.442	0.437	0.426	0.450	0.438	0.990	0.960	[illegible]	0.440	0.419	0.412	0.424	0.933	6.661	.066

multiply by 100 to get %.

control here is O.D. value (mean) w/ GTC-buffer [raw data - row C]

control here is O.D. value ~~(mean)~~ w/ heated buffer (raw data - row E)

Value highlighted in pink has been changed from raw data due to very high repeating O.D.'s - (higher than control) used the mean of no competitor control data (raw data in row A).

% reduction is $100 - (100 \times \frac{\text{mean}}{\text{control}})$

When "w/o compet" (raw data row A) is used as the control to calc % reduction, I get the following values.

1B6 - 12.89	5C5 - 41.5
1E7 - 39.9	6F12 - 21.5
2E6 - 11.6	7C10 - 22.3
3B3 - 32.1	9D5 - 3.5
3E3 - 15.6	
4E12 - 37.6	
4F12 - 30.4	

See plotted data next pg.

This same control could probably be used for each mAb → (the row A data - no competitor) since the heated buffer control values appear to fluctuate a bit. These calculations are listed in the column - written by hand.

Signed Heidi Sleister Date

Witnessed Teresa Thompson Date

contd Data from prev. pages – Histogram

Date

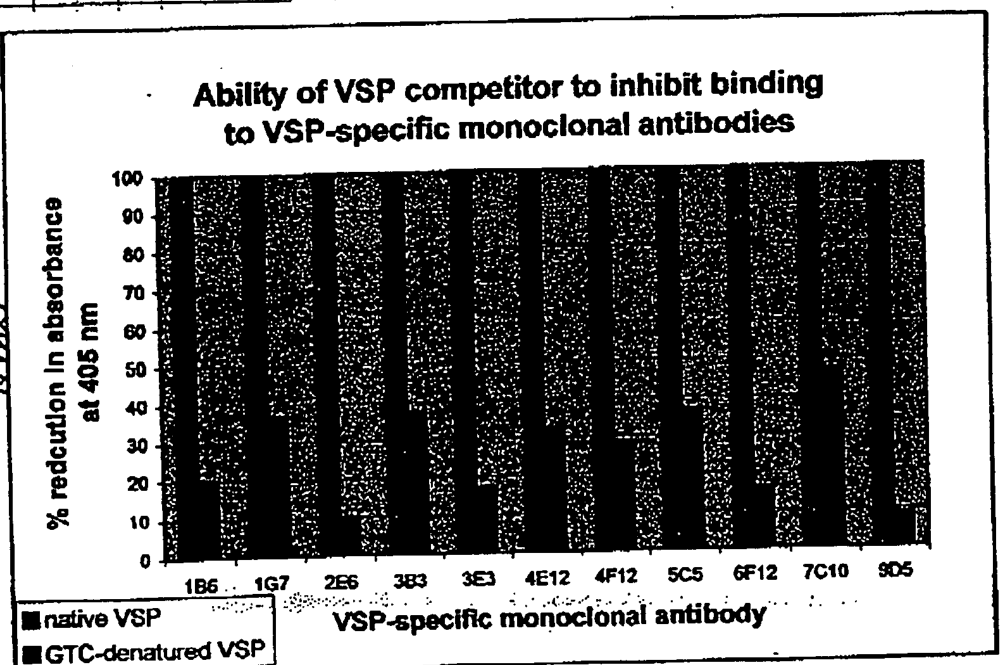
**Note- the actual value for native VSP below was normalized to 100, and the values for GTC- and heat-denatured VSP were compared to these.

	% reduction in absorbance at 405 nm			
mAb	actual value native VSP	normalized native VSP	actual value GTC-VSP	comparitive GTC-denatured VSP
1B6	60.925	100.000	12.169	19.974
1G7	64.358	100.000	23.626	36.711
2E6	54.595	100.000	5.544	10.155
3B3	62.062	100.000	23.346	37.617
3E3	60.213	100.000	10.408	17.286
4E12	65.091	100.000	21.097	32.412
4F12	49.360	100.000	14.168	28.704
5C5	77.763	100.000	28.761	36.990
6F12	74.346	100.000	11.761	15.819
7C10	66.453	100.000	31.226	46.989
9D5	10.851	100.000	0.980	9.035

% reduction w/native VSP compet

% reduction w/GTC-denatured VSP

HMD



HMD

Comments: When displayed as a histogram, it is obvious that native VSP acts as a competitor in FACE ~~for~~ biotinylated VSP binding to VSP-specific mAbs. In contrast, GTC-denatured VSP does not act as a good competitor. Therefore, it is likely that the VSP-specific mAbs recognize native, conformational epitopes on VSP.

Signed Heidi Fleister Date

Witnessed Teresa Thompson Date

EXPERIMENT 3

Appl. No. 09/478,598
Filed: January 6, 2000

Determination of the additivity index: to test the ability of two monoclonal antibodies to bind simultaneously on the antigen (Ref: Friguet et al, Molecular Immunology, Vol. 21, pp 673-677, 1984).

Procedure: the antibodies are added separately and together, at saturating concentration, to the coated antigen on a microtiter plate. The amount of bound antibodies is then quantitated by the usual indirect ELISA.

MONOCLONAL 1 (Twice saturated Concn.)

1 ml

0.5 ml — 0.5 ml

MONOCLONAL 2 (Twice saturated Concn.)

1 ml.

0.5 ml — 0.5 ml.

+0.5 ml buffer to give satd concn → 200 ul to antigen coated plate

MIX — Each monoclonal is now at saturated concn → 200 ul to antigen coated plate

+0.5 ml buffer to give satd concn → 200 ul to antigen coated plate

This process is repeated for "pairs" of monoclonals in a matrix system as described on subsequent pages.

Witnessed

Date

Date

Continued from previous page

Preparation of Twice saturated monoclonals in PBS (2.5 ml)

III H11	→	500 ul
IV F10	→	12.5 ul.
III B3	→	12.5 ul
III E3	→	12.5 ul of 1:4 diluted stock
I B6	→	12.5 ul
I D5	→	10.5 ul
VII C10	→	12.5 ul of 1:4 diluted stock
II E6	→	12.5 ul of 1:4 " "
VII B9	→	100 ul ~~of~~
V C5	→	10.5 ul
I G7	→	7.0 ul.

These calculations were made based on the saturation curves described on pages 058 – 063 of this notebook.

500 ul samples of "paired" monoclonals stocks were prepared according to a grid shown on the next page, using eppendorf tubes.

Witnessed Larry R Bech

A. Gurunay Ray Date

Date

	ⅢH11	IVF10	ⅢB3	ⅡE3	IB6	ID5	VIIC10	ⅡE6	VIIB9	VC5	IG7
ⅢH11	+buffer	+	+	+	+	+	+	+	+	+	+
ⅣF10		+buffer	+	+	+	+	+	+	+	+	+
ⅢB3			+buffer	+	+	+	+	+	+	+	+
ⅢE3				+buffer	+	+	+	+	+	+	+
IC6					+buffer	+	+	+	+	+	+
ID5						+buffer	+	+	+	+	+
VIIC10							+buffer	+	+	+	+
ⅡE6								+buffer	+	+	+
VIIB9									+buffer	+	+
VC5										+buffer	+
IG7											+ buffer

From these samples, 200 ul aliquots were dispensed in duplicate to antigen coated microtiter plates, according to the matrix system shown on the next page

Witnessed Larry R. Beck Date

A. Gururaj Rao Date

Microtiter plate format

Plate I

		1	2	3	4	5	6	7	8	9	10	11	12
		BLANK	III H11	IV F10	III B3	III E3	I B6	I D5	VII C10	II E6	VII B9	V C5	I E7
III H11	A		—										
	B		—										
IV F10	C			—									
	D			—									
III B3	E				—								
	F				—								
III E3	G					—							
	H					—							

Plate II

		1	2	3	4	5	6	7	8	9	10	11	12
		BLANK	I B6	I D5	VII C10	II E6	VII B9	V C5	I E7				
I B6	A		—										
	B		—										
I D5	C			—									
	D			—									
VII C10	E				—								
	F				—								
II E6	G					—							
	H					—							

Plate III

		1	2	3	4	5	6	7	8	9	10	11	12
		BLANK	VII B9	V C5	I E7								
VII B9	A		—										
	B		—										
V C5	C			—									
	D			—									
I E7	E				—								
	F				—								
	G												
	H												

— indicates monoclonals diluted with buffer. All other wells contain paired monoclonal antibodies.

ELISA's were done as before and A_{405} measurements made every 10 min up to 100 min

Witnessed [signature]
[signature] Date
Date

Plate I — OPTICAL DENSITY — 100 min

		1	2	3	4	5	6	7	8	9	10	11	12
		Blnk	III H11	IVA10	III B3	III E3	IB6	IDS	VIIC10	IIE6	III B9	VCS	I67
III H11	A	0.073	0.234	0.370	0.372	0.581	0.399	0.376	0.569	0.559	0.451	0.364	0.411
	B	0.056	0.240	0.370	0.379	0.622	0.419	0.398	0.599	0.595	0.427	0.341	0.366
NA10	C	0.062	0.069	0.319	0.331	0.590	0.373	0.323	0.580	0.550	0.440	0.305	0.350
	D	0.063	0.063	0.313	0.313	0.583	0.349	0.316	0.559	0.557	0.442	0.319	0.359
III B3	E	0.065	0.061	0.058	0.775	0.562	0.339	0.335	0.560	0.580	0.444	0.377	0.344
	F	0.058	0.060	0.059	0.058	0.062	0.069	0.064	0.072	0.067	0.072	0.064	0.065
III E3	G	0.059	0.064	0.059	0.058	0.551	0.577	0.591	0.531	0.594	0.376	0.604	0.621
	H	0.058	0.078	0.065	0.051	0.574	0.677	0.635	0.595	0.501	0.606	0.651	0.709

Plate II — OPTICAL DENSITY — 100 min

		1	2	3	4	5	6	7	8	9	10	11	
				IDS	VIIC10	IIE6	VIIB9	VCS	I67				
IB6	A	0.061	0.265	0.338	0.505	0.506	0.400	0.299	0.371	0.036	0.039	0.038	0.
	B	0.061	0.251	0.322	0.472	0.473	0.362	0.292	0.354	0.036	0.036	0.034	0.
IDS	C	0.060	0.058	0.268	0.484	0.482	0.401	0.319	0.300	0.040	0.038	0.036	0.
	D	0.059	0.062	0.269	0.481	0.481	0.349	0.334	0.307	0.037	0.039	0.039	0.
VIIC10	E	0.059	0.064	0.066	0.447	0.452	0.436	0.464	0.521	0.038	0.039	0.038	0.
	F	0.064	0.059	0.057	0.448	0.467	0.460	0.464	0.537	0.038	0.036	0.037	0.
IIE6	G	0.061	0.061	0.063	0.062	0.452	0.466	0.540	0.569	0.037	0.037	0.037	0.
	H	0.061	0.060	0.061	0.072	0.464	0.487	0.457	0.526	0.042	0.037	0.038	0.

Plate III — OPTICAL DENSITY — 100 min

		1	2	3	4	5	6	7	8	9	10	11	12
			VIIB9	VCS	I67								
VIIB9	A	0.063	0.259	0.381	0.453	0.063	0.061	0.059	0.055	0.052	0.057	0.059	0.055
	B	0.066	0.250	0.375	0.440	0.056	0.059	0.055	0.060	0.060	0.058	0.056	0.056
VCS	C	0.066	0.055	0.382	0.377	0.057	0.058	0.052	0.055	0.062	0.058	0.056	0.057
	D	0.078	0.069	0.297	0.378	0.058	0.053	0.057	0.057	0.061	0.060	0.060	0.061
I67	E	0.072	0.071	0.074	0.342	0.054	0.063	0.059	0.062	0.063	0.060	0.055	0.062
	F	0.075	0.076	0.074	0.351	0.058	0.066	0.065	0.066	0.069	0.069	0.071	0.068
	G	0.082	0.055	0.079	0.083	0.065	0.064	0.069	0.066	0.064	0.067	0.067	0.065
	H	0.083	0.080	0.077	0.075	0.067	0.064	0.068	0.067	0.080	0.070	0.072	0.069

Witnessed [illegible signature] Date

[illegible signature] Date

Calculation of the additivity Index (AI)

The additivity index AI is defined as a term to quantify the ability of two antibodies to bind simultaneously onto the antigen:

$$A.I. = \frac{(A1+2) - \frac{A1+A2}{2}}{\frac{A1+A2}{2}} \times 100$$

where $A1$ = A_{405} in the well with first antibody alone

$A2$ = A_{405} " " " " second " "

$(A1+2)$ = A_{405} in the well with 2 antibodies mixed together.

This formula was used to calculate AI for pairs of monoclonals, after subtracting the blank A_{405} from the lanes marked blank.

Example: III H11 + IV F10, lane 3 plate I

A1 (absorbance of III H11 alone) $= \left(\frac{0.234+0.240}{2} = 0.237\right) - \text{Blank}$

$= 0.237 - 0.070$

$= 0.167$

A2 (absorbance of IV F10 alone) $= \left(\frac{0.319+0.313}{2} = 0.316\right) - \text{Blank}$

$= 0.316 - 0.066$

$= 0.250$

(A1+2) (absorbance of III H11 + IV F10, lane 3) $= 0.370 - 0.070 = 0.300$

Witnessed Gary R. Beale
R. Gururaj Rao
Date
Date

A·I for III H11 + IV F10

$$= \frac{0.300 - \frac{0.167 + 0.250}{2}}{\frac{0.167 + 0.250}{2}} \times 100$$

$$= \frac{0.300 - 0.209}{0.209} \times 100 = \frac{0.091}{0.209} \times 100$$

$$= 44$$

A·I's were similarly calculated for all the other pairs

ADDITIVITY INDEX

	IIIH11	IVF10	IIIB3	IIE3	IB6	ID5	VIIC10	IIE6	VIIB9	VC5	IG7
IIIH11	—	44	*30*	60	84	67	86	80	106	45	49
IVF10		—	*2*	41	*34*	*13*	60	52	73	*6*	*12*
IIIB3			—	*26*	*12*	48	46	50	*12*	*21*	*20*
IIIE3				—	55	55	*12*	*19*	72	57	55
IB6					—	*33*	47	44	65	*12*	*28*
ID5						—	42	40	56	*17*	*1*
VIIC10							—	*2*	*34*	47	40
IIE6								—	41	40	54
VIIB9									—	53	56
VC5										—	*25*
IG7											—

The bolded & italicized numbers indicate antibody pairs possibly recognizing common epitopes.

Witnessed [signature] Date

[signature] Date

Antibody pairs possibly recognizing same epitope based on A.I. values

~~III B3 - III H11~~
III B3 - IV F10
III B3 - III E3
III B3 - I B6.
III B3 - VII B9
III B3 - V C5
III B3 - I G7

IV F10 — I B6.
IV F10 - I D5
IV F10 - V C5
IV F10 — I G7
I B6 - I D5
I B6 - V C5
I B6 - I G7.

III E3 - VII C10
III E3 - II E6
I D5 - V C5
I D5 - I G7.
VII C10 - II E6
V C5 - I G7.

ADDITIVITY INDEX

(data from experiment of recalculated and corrected)

	IIIH11	IVF10	IIIB3	IIIE3	IB6	ID5	VIIC10	IIE6	VIIB9	VC5	IG7
IIIH11	—	44	50	60	72	67	86	80	106	45	49
IVF10		—	2	41	34	13	60	52	73	6	12
IIIB3			—	26	12	48	46	50	78	21	20
IIIE3				—	55	55	12	19	53	50	55
IB6					—	33	47	44	65	12	28
ID5						—	42	40	56	17	1
VIIC10							—	2	34	47	40
IIE6								—	41	40	54
VIIB9									—	53	65
VC5										—	25
IG7											—

Witnessed [signature] Date

[signature] Date

Repeat of additivity index experiment described on pages 070 → 077

ADDITIVITY INDEX

(data from experiment of)

	IIIH11	IVF10	IIIB3	IIIE3	IB6	ID5	VIIC10	IIE6	VIIB9	VC5	IG7
IIIH11	—	48	56	50	50	61	61	68	75	55	63
IVF10		—	3	34	14	8	40	46	72	9	23
IIIB3			—	29	17	10	50	49	70	29	27
IIIE3				—	31	43	2	12	40	40	44
IB6					—	23	40	40	64	13	34
ID5						—	40	50	68	23	15
VIIC10							—	4	40	40	49
IIE6								—	42	37	50
VIIB9									—	61	82
VC5										—	32
IG7											—

ADDITIVITY INDEX

(data from experiment of top line and experiment of bottom line)

	IIIH11	IVF10	IIIB3	IIIE3	IB6	ID5	VIIC10	IIE6	VIIB9	VC5	IG7
IIIH11	—	44 48	50 56	60 50	72 50	67 61	86 61	80 68	106 75	45 55	49 63
IVF10		—	2 3	41 34	34 14	13 8	60 40	52 46	73 72	6 9	12 23
IIIB3			—	26 29	12 17	48 10	46 50	50 49	78 70	21 29	20 27
IIIE3				—	55 31	55 43	12 2	19 12	53 40	50 40	55 44
IB6					—	33 23	47 40	44 40	65 64	12 13	28 34
ID5						—	42 40	40 50	56 68	17 23	1 15
VIIC10							—	2 4	34 40	47 40	40 49
IIE6								—	41 42	40 37	54 50
VIIB9									—	53 61	65 82
VC5										—	25 32
IG7											—

Witnessed

Date

Date

70 minute time point readings of plates I, II & III

Plate I **OPTICAL DENSITY** 70 min

		1	2	3	4	5	6	7	8	9	10	11	12
		BLNK	IIIH11	IVF10	IIIB3	IIE61	IB6	IDS	VIIC10	IIE6	VIIB9	VCS	IG7
IIIH11	A	0.045	0.168	0.252	0.254	0.360	0.248	0.249	0.350	0.347	0.252	0.235	0.260
	B	0.044	0.161	0.251	0.250	0.362	0.246	0.251	0.347	0.350	0.251	0.240	0.263
IVF10	C	0.048	0.044	0.204	0.204	0.344	0.228	0.203	0.335	0.245	0.284	0.203	0.238
	D	0.045	0.046	0.203	0.203	0.336	0.214	0.204	0.333	0.326	0.281	0.206	0.227
IIIB3	E	0.042	0.045	0.042	0.195	0.340	0.228	0.199	0.317	0.347	0.266	0.231	0.223
	F	0.042	0.049	0.042	0.161	0.320	0.213	0.188	0.320	0.324	0.256	0.222	0.219
IIIE3	G	0.046	0.038	0.040	0.033	0.320	0.333	0.307	0.284	0.291	0.337	0.326	0.358
	H	0.042	0.039	0.037	0.037	0.368	0.351	0.356	0.325	0.350	0.337	0.344	0.336

Plate II **OPTICAL DENSITY** 70 min

		1	2	3	4	5	6	7	8	9	10	11	12
		BLNK	IB6	IDS	VIIC10	IIE6	VIIB9	VCS	IG7				
IB6	A	0.054	0.220	0.245	0.339	0.343	0.272	0.223	0.257	0.034	0.034	0.034	0.033
	B	0.056	0.205	0.229	0.322	0.326	0.252	0.210	0.245	0.032	0.037	0.033	0.032
IDS	C	0.047	0.051	0.184	0.323	0.335	0.270	0.216	0.211	0.031	0.032	0.030	0.031
	D	0.050	0.047	0.181	0.311	0.338	0.254	0.214	0.213	0.030	0.028	0.026	0.029
VIIC10	E	0.047	0.046	0.049	0.234	0.304	0.306	0.318	0.343	0.029	0.031	0.026	0.028
	F	0.047	0.043	0.045	0.315	0.313	0.302	0.317	0.342	0.027	0.027	0.028	0.028
IIE6	G	0.042	0.042	0.043	0.058	0.292	0.305	0.281	0.317	0.028	0.027	0.027	0.029
	H	0.043	0.042	0.041	0.044	0.045	0.314	0.312	0.365	0.059	0.032	0.028	0.027

Plate III **OPTICAL DENSITY** 70 min

		1	2	3	4	5	6	7	8	9	10	11	12
		BLNK	VIIB9	VCS	IG7								
VIIB9	A	0.072	0.190	0.279	0.311	0.040	0.042	0.041	0.043	0.048	0.042	0.042	0.041
	B	0.057	0.192	0.272	0.305	0.041	0.042	0.041	0.045	0.041	0.044	0.046	0.041
VCS	C	0.059	0.066	0.201	0.251	0.042	0.040	0.040	0.043	0.036	0.047	0.040	0.039
	D	0.050	0.061	0.192	0.233	0.041	0.040	0.041	0.044	0.043	0.041	0.041	0.042
IG7	E	0.061	0.047	0.063	0.201	0.041	0.042	0.043	0.042	0.042	0.041	0.044	0.041
	F	0.048	0.060	0.055	0.210	0.041	0.041	0.040	0.039	0.039	0.041	0.042	0.043
	G	0.062	0.059	0.055	0.059	0.042	0.042	0.041	0.044	0.050	0.047	0.046	0.056
	H	0.062	0.059	0.059	0.057	0.038	0.041	0.043	0.038	0.038	0.042	0.048	0.049

Witnessed [signature] Date

[signature] Date

EXPERIMENT 4

Appl. No. 09/478,598
Filed: January 6, 2000

VSP-specific
mAb IIIE3 recognizes VSP (from soybean) on a Western Blot

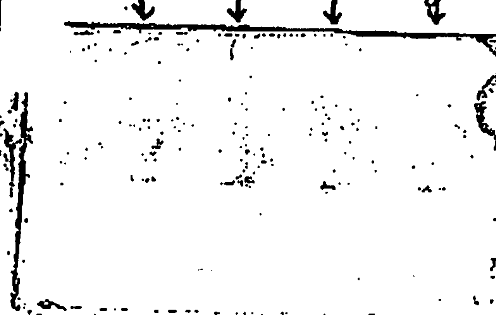
Book 93 p. 100

Purpose: To identify which VSP-specific mAb recognizes VSP on a Western blot.

+ Last week, Keith found that of 3 pools of ~~6~~ 10 VSP-specific mAbs, one pool contained ≥ 1 Ab recognizing VSP on a Western. This pool contained mAbs VC5, IIIE3, IVF10, VIIC10. VC5 was present also in the negative result pools, so this limits possibilities to IIIE3, IVF10, & VIIC10.

Ran 1γ soybean VSP in 4 separate lanes of a 12% tris-glycine SDS-PAGE - no DTT added to sample buffer. - 200V. ~35 min.

1γ VSP per lane (↓) - no DTT added to sample buf



post-transfer gel

Blotted proteins on gel to PVDF membrane as described on p.100 book (2 hr. transfer). Rinsed blot 1X in PBS + inc. o/n w/ PBS - 3% BSA (2.5 ml). (Scored blot to cut in 4 pcs. before rinsing w/ PBS).

Cut blot in 4 strips. Stained & destained post-transfer gel. Added 1° Ab (5 ml) to each strip separately -

Strip 1 - mAb IIIE3
2 - " IVF10
3 - " VIIC10
4 - polyclonal Ab

VSP-specific Abs

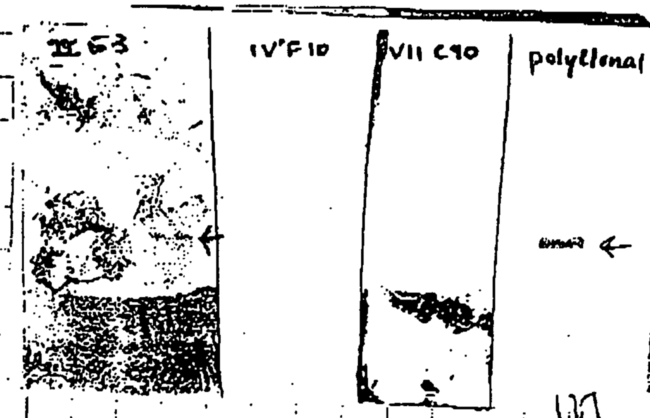
used following ants Ab

1° Ab - 1hr
washed 4x5' PBS
2° Ab - (goat anti-mouse IgG) 1:7,500 - 10 ml per strip
Washed 4x PBS (5'), 1x PBS - .05% tween (5')
Added developer -
(100 mM NaCl, 5 mM MgCl₂, 100 mM Tris pH 9.5)
added to 15 ml - 99 μl 5% NBT + 50 λ 5% BCIP

IIIE3 - 10 μl @ 3 ng/ml in 5 ml → 6 ng/ml
IV F10 - 5 μl @ .09 mg/ml " → .548 μg/ml
VIIC10 - 10 μl @ .43 mg/ml " → .868 μg/ml
polyclonal - clump of cells into 10 ml PBS-BSA

final conc in PBS-BSA

dev'd ~40 min.



CONCL: mAb IIIE3 reacts w/ VSP on a Western blot, so only 1 (out of 10) mAbs tested work on W. blot.

Witnessed Heidi Slisger

mAbs tested: IB6, IG7, III B3, IIIE3, IV E12, IV F10, IV F12, V C5, VI F12, VII C10

EXPERIMENT 5

Appl. No. 09/478,598
Filed: January 6, 2000

ELISA to test foldedness of recombinant Met 10 (by binding to conformational monoclonal antibodies)...

* Note - this ELISA was done last wk

*Purpose: VSPβ-met 10 was designed in hopes of retaining Met 10 structure. To test this, recombinant Met 10 (refolded from Ni-NTA-purified Baculovirus inclusion bodies) was monitored for its ability to bind monoclonal antibodies that recognize native soybean VSP (i.e. - conformational mAbs).

LW

5 μg/ml

		1	2	3	4	5	6	7	8	9	10	11	12
		TEMPLATE		1B6 / 4F12	1G5 / 5C5	1G7 / 6F12	2E6 / 7C10	3B3 / 9D5	3E3 / 1:5K	4E10 / 1:5K	4F10 / 1:5K		
Bac WT	A	X		1B6	1G5	1G7	2E6	3B3	3E3	4E10	4F10		
	B			4F12	5C5	6F12	7C10	9D5	1:5K serum mouse R	1:5K SSL ser	1:5K SDH serum		
Bac met 10	C	X		1B6									
	D			4F12									
∅	E												
	F												
soy	G												
	H		X										

METHODS: Followed ELISA protocol as described on p. 30 - used 5 μg/ml of each antigen - immob. on Nunc maxisorb plt. @ 4°C O/N. Blocked O/N w/ 3% BSA in PBS - O/N 4°C

Inc. washed plt w/ mAbs (used 5 μg each except 9D5 - 2 μg, 2E6 - 1 μg, 1G5 - 0.5 μg) Inc 37°C 2 hrs.

1:10K dil anti-mouse IgGγ-biotin conj.

1:40K dil extravidin-AP

Controls: ⊕ 1:5,000 dilution anti-VSPβ-met 10/20 serum (mouse R) 1st.

⊕ 1:5,000 dil. anti-soybean VSP serum (mouse L) V of 1A

⊖ 1:5,000 dil anti-SDH serum (mouse 126 - V of 1A)

Also rows E + F are no antigen neg. controls.

Results: I could see positive signals within minutes of adding the substrate - see next pg.

Signed Heidi Steister Date Witnessed Susan Grant Date

45 covered Results

Date

* Results obtained

11 min

		1	2	3	4	5	6	7	8	9	10	11	12
Bac WT	A	0.072	0.073	0.193	0.148	0.197	0.204	0.236	0.317	0.207	0.191	0.073	0.073
	B	75	74	152	154	224	198	200	197	200	83	74	74
Bac mer10	C	0.073	0.07[illegible]	0.146	0.082	0.089	0.089	0.087	0.230	0.111	0.115	0.075	0.079
	D	0.072	0.073	0.100	0.126	0.190	0.189	0.133	0.207	0.164	0.082	0.073	0.067
φ	E	0.072	0.072	0.089	0.080	0.077	0.079	0.086	0.139	0.090	0.087	0.073	0.073
	F	0.071	0.072	0.094	0.078	0.105	0.157	165	74	87	71	74	73
SOY VSP	G	72	72	180	160	175	208	206	190	238	211	75	73
	H	0.072	0.072	0.155	0.178	0.226	0.194	0.205	0.143	0.232	0.082	0.078	0.074

WMO

40 min

		1	2	3	4	5	6	7	8	9	10	11	12
Bac WT	A	0.073	0.074	0.609	0.376	0.441	0.520	0.500	0.533	0.493	0.456	0.074	0.073
	B	0.075	0.074	0.425	0.408	0.555	0.613	0.502	0.451	0.512	0.104	0.074	0.074
Bac mer10	C	0.074	0.073	0.439	0.116	0.145	0.141	0.161	0.530	0.192	0.213	0.079	0.074
	D	0.073	0.074	0.207	0.230	0.576	0.443	0.261	0.491	0.404	0.098	0.074	0.082
φ	E	0.073	0.073	0.193	0.107	0.092	0.099	0.096	0.272	0.095	0.119	0.074	0.073
	F	0.072	0.073	0.162	0.094	0.194	0.310	0.155	0.077	0.132	0.082	0.071	0.074
SOY VSP	G	0.073	0.073	0.543	0.424	0.450	0.516	0.475	0.432	0.526	0.461	0.075	0.074
	H	0.074	0.074	0.425	0.460	0.647	0.510	0.512	0.286	0.539	0.098	0.079	0.075

Formula: L1

WMO

80 min

		1	2	3	4	5	6	7	8	9	10	11	12
Bac WT	A	0.073	0.074	0.786	0.523	0.643	0.759	0.626	0.720	0.661	0.074	0.073	0.000
	B	0.076	0.075	0.544	0.555	0.910	0.792	0.675	0.700	0.119	0.074	0.075	0.000
mer10	C	0.075	0.074	0.520	0.127	0.178	0.161	0.200	0.257	0.287	0.087	0.074	0.000
	D	0.073	0.075	0.245	0.284	0.765	0.612	0.190	0.545	0.113	0.073	0.121	0.000
φ	E	0.074	0.073	0.251	0.114	0.103	0.106	0.647	0.109	0.148	0.073	0.074	0.000
	F	0.072	0.073	0.199	0.105	0.257	0.438	0.726	0.175	0.092	0.071	0.075	0.000
SOY VSP	G	0.074	0.074	0.749	0.550	0.625	0.767	8.000	0.736	0.669	0.074	0.074	0.000
	H	0.075	0.075	0.578	0.619	0.932	0.747	0.747	0.794	0.112	0.077	0.075	0.000

WMO

Formula: L1

Heidi Louisaur

Date

Susan Grant

Date

cont'd
Results

Date

Looking @ 40 min time pt -

Clones (mAbs) that look best (not much dif. between Met10 + WT) are 6F12, 7C10, 1B6, 3E3.
Even at t=80 min, 1G5 looks negative (0.127 compared to 0.523 (Bac-WT) or 0.550 (VSP soy native) + 0.073 (neg control - SDH serum).

The values for native soybean VSP (rows G, H) + Baculovirus recombinant VSPβ-WT (rows A + B) are very similar in terms of readings - GREAT!

I'm not sure why the readings in wells 9B, 9D, 9F, 9H are lower after 80 min compared to 40 min!!? Also, the reading of 8.0 in well G7 must have been caused by a bubble?

Nearly all of the mAbs recognize refolded Met10. However, the values for Met10 in many cases are lower than WT (eg - vs. mAb 7C10 - well 6B (.613) vs 6D (.443). Met10 is clearly positive for binding 7C10 (compare 6D @ .443 w/ neg control 10D @ .098) - yet WT gives a higher rdg. (.613).

→ Conclusion - although this ELISA is not quantitative, the clear conclusion is that nearly all the conformational mAbs recognize refolded Met10 - suggesting that this Met-enriched VSPβ variant may be correctly folded.

* Note - the background values in rows E + F suggest that the antibody concentration was too high (used 5μg/well). I'll repeat this ELISA using less mAb (0.25μg) - see next pg. Also - I can try a dilution series ELISA (add dil. series of the mAb).

Signed Heidi Kreisher ______ Date

Witnessed Susan Grant ______ Date

repeat → ELISA to test foldedness of Met 10 (refolded from Incl. bodies - 47 recombinant expression in Baculovirus)

Date

Purpose: This ELISA is the same as described on pp 44-46, except some incubation times differ + here I used 0.25µg/well of each mAb (previously used 5µg).

Methods:
Immob. 5µg/ml antigen (in PBS) to wells as shown on template - p. 44. Inc O/N 4°C. Blocked 2.5 hrs @ 37°C w/ BSA.
Antibodies
1:10K dil anti-mouse IgG γ-biotin conj - 90 min 37°C
1:50K dil extravidin AP - 30 min RT

mAb	conc	µl for 1µg	400µl = µl Ab.
1B6	.39 mg/ml	2.6	397.4
1G5	.011	90.9	309.1
1G7	.585	1.7	398.3
2E6	.022	45.5	354.5
3B3	.587	1.7	398.3
3E3	.096	10.4	389.6
4E10	1.27	.79	399.2
4F10	.324	3.1	396.9
4E12	.501	2	398
5C5	.278	3.6	396.4
6F12	.282	3.5	396.5
7C10	.430	2.3	397.7
9D5	.048	20.8	379.2

HS

Added 0.25µg each mAb/well see template - p. 44.

Added substrate (PNPP in diethanolamine buffer) @ 2:58 pm.

The yellow was slower to come up this time - but I still saw positives in rows A, B, G, H within a few minutes (~5-10 min)

120 min

		1B6	1G5	1G7	2E6	3B3	3E3	4E10	4F10		
		4F12	5C5	6F12	7C10	9D5	serum ⊕	serum ⊕	⊖ serum		
Bac WT		[illegible]	0.372	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bac met 10	72	96	79	82	81	78	314	78	95	71	72
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
no antigen		[illegible]	0.074	0.075	[illegible]	[illegible]	0.147	[illegible]	[illegible]	[illegible]	0.072
	0.072 0.076	0.074	0.077	0.097	0.146	0.089	0.075	[illegible]	[illegible]	[illegible]	[illegible]
8oz VSP		0.822	0.607	[illegible]	[illegible]	596	832	496	565	72	71
		372	470	667	550	495	366	827	57	74	74

HS

After ~130 min, put plate @ -20°C to stop rxn - O/N
I'll thaw + incubate longer tomorrow.

Signed Heidi Sleister Date

Witnessed Susan Grant Date

contd

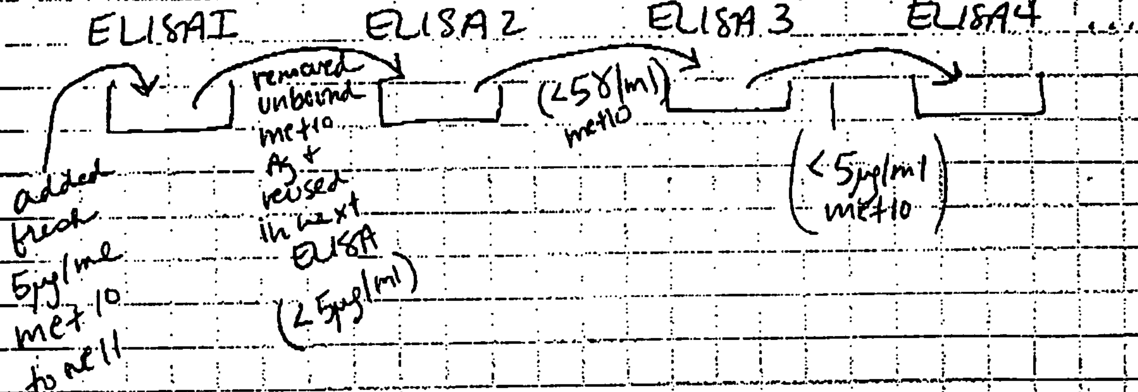
Date

- Thawed plt + inc. @ RT. (Removed from freezer ~9:10 AM

The positives after 120 min are 6F12, 7C10, 3E3. The values are lower than those observed yesterday (p.45). Even antibody 3E3 which we would expect to give equal values for WT + met10 (because it's thought to recognize ~~a~~ denatured VSP ~~epitope~~) gives a 3-fold higher reading w/ WT in this ELISA

The antigen used (WT + met10) have been used various numbers of times. For example, the WT antigen was fresh this ELISA + the met10 antigen has been used repeatedly (~5-6 times). In effect, there could be less met10 immobilized to the wells compared to WT.

* Further evidence - the pos. control serum in wells 8B + 8D was from a mouse immunized w/ met 10/20 + therefore we'd expect a higher reading in well 8D (.588) than 8B (.752). We see the opposite. One possibility for this is Met 10 conc. on the well is lower than WT.



Signed Heidi Sleister Date

Witnessed Susan Grant Date

51 ELISA to test foldedness of Met 10 (refolded from Baculovirus inclusion bodies)

ELISA: mAb Serial dilution

Date

Purpose: Same exp. as on p. 47, except here using serial dil's of mAb's + fresh antigen.

- Coated plt w/ freshly made (diluted) antigen @ 2.5 μg/ml Inc ON 4°C.
- Blocked 2 hrs @ 37C., washed 3X PBST.
- Added mAb as indicated - in serial dilutions. (See labels on top panel of data - p. 52 + 54. Added 2.5 μg of mAb to each well in row B + serially diluted 2-fold going down the column, except for mAb 3E3 which had 0.5 μg added to wells 3B + 4B + 2-fold serial dil's for the rest of columns 3 + 4. Note! no mAb was added to row A - this is a blank. Positive control serum was added to cols. 11 + 12. Inc mAb's 2 hrs @ 37°C.

Cont'd w/ remainder of ELISA exactly as described on p. 57.

Met 10 was coated in columns 1, 3, 5, 7, 9, 11
WT was " " " 2, 4, 6, 8, 10, 12
Both antigens are from Baculovirus - intracellular proteins.

mAbs added -

Plate	Columns	mAb
Plate A	1,2	7C10
	3,4	3E3
	5,6	6F12
	7,8	1B6
	9,10	4F10
	11,12	1G7
Plate B	1,2	3B3
	3,4	4E12
	5,6	4F12
	7,8	5C5
	9,10	6F12
	11,12	serum mouse 55L

Signed Heidi Sleister Date Witnessed Teresa Thompson Date

contd Plate A Results

Date

Plate 3A - 21 min — Plate#1

	7C10		mAb 3E3		6F12		1B6		4F10		1G7		Row A
	1	2	3	4	5	6	7	8	9	10	11	12	
	met lo	wt	met lo	wt	met lo	wt	met lo	wt	met lo	wt	met lo	wt	
A	0.071	0.072	0.073	0.072	0.072	0.072	0.072	0.072	0.072	0.071	0.072	0.072	← Blank
B	0.190	0.247	0.205	0.252	0.235	0.307	0.181	0.282	0.118	0.223	0.083	0.191	2.5γ mAb
C	0.208	0.242	0.190	0.252	0.187	0.293	0.130	0.278	0.099	0.215	0.077	0.188	1.25γ
D	0.202	0.219	0.172	0.239	0.148	0.237	0.103	0.229	0.086	0.186	0.073	0.197	.63γ
E	0.172	0.194	0.138	0.188	0.116	0.224	0.090	0.217	0.080	0.183	0.071	0.184	.31γ
F	0.149	0.164	0.115	0.155	0.096	0.194	0.081	0.191	0.076	0.180	0.070	0.187	.16γ
G	0.143	0.147	0.098	0.141	0.085	0.193	0.077	0.179	0.073	0.178	0.071	0.183	.08γ
H	0.116	0.122	0.088	0.114	0.081	0.159	0.075	0.174	0.073	0.185	0.078	0.179	.04γ

IAP3 HRP

Plate 3A - 45 min — Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.072	0.073	0.074	0.073	0.073	0.072	0.072	0.072	0.072	0.072	0.072	0.072
B	0.375	0.443	0.368	0.444	0.381	0.517	0.265	0.450	0.158	0.364	0.091	0.305
C	0.379	0.446	0.309	0.435	0.292	0.485	0.180	0.446	0.119	0.340	0.081	0.292
D	0.364	0.402	0.281	0.423	0.220	0.407	0.130	0.378	0.098	0.301	0.075	0.284
E	0.365	0.383	0.222	0.316	0.163	0.345	0.105	0.341	0.087	0.280	0.072	0.286
F	0.308	0.312	0.166	0.249	0.124	0.312	0.089	0.294	0.081	0.271	0.071	0.273
G	0.243	0.241	0.130	0.205	0.099	0.294	0.082	0.263	0.075	0.247	0.071	0.272
H	0.184	0.186	0.116	0.164	0.091	0.259	0.079	0.271	0.077	0.277	0.081	0.268

IAP4 HRP

Plate 3A - 75 min — Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.073	0.074	0.077	0.068	0.072	0.073	0.073	0.073	0.073	0.072	0.073	0.073
B	0.531	0.612	0.498	0.635	0.545	0.736	0.348	0.653	0.210	0.491	0.104	0.413
C	0.538	0.635	0.427	0.622	0.412	0.679	0.244	0.617	0.144	0.472	0.088	0.415
D	0.499	0.576	0.379	0.589	0.300	0.581	0.173	0.532	0.114	0.422	0.078	0.399
E	0.502	0.536	0.288	0.445	0.222	0.514	0.129	0.491	0.098	0.401	0.074	0.410
F	0.430	0.439	0.222	0.352	0.158	0.476	0.102	0.445	0.082	0.385	0.072	0.413
G	0.346	0.326	0.160	0.272	0.117	0.412	0.089	0.373	0.078	0.378	0.073	0.417
H	0.254	0.262	0.137	0.222	0.104	0.375	0.084	0.396	0.079	0.391	0.081	0.415

For a graph of this data, see nb# 5416, p.73-75

Signed Heidi Bleister Date Witnessed Lisa Thompson, Date

53 cont'd - Plate A

Date

Plate 3A - 110 min

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.073	0.078	0.079	0.069	0.074	0.074	0.074	0.074	0.073	0.073	0.074	0.074
B	0.617	0.721	0.578	0.752	0.678	0.938	0.448	0.852	0.258	0.642	0.116	0.536
C	0.618	0.766	0.520	0.795	0.546	0.901	0.303	0.840	0.170	0.621	0.095	0.541
D	0.587	0.685	0.472	0.749	0.387	0.762	0.211	0.716	0.134	0.557	0.082	0.528
E	0.564	0.626	0.360	0.578	0.272	0.687	0.150	0.651	0.110	0.524	0.077	0.532
F	0.487	0.510	0.267	0.442	0.189	0.608	0.115	0.580	0.096	0.503	0.074	0.534
G	0.395	0.377	0.181	0.319	0.131	0.525	0.096	0.490	0.081	0.488	0.074	0.544
H	0.291	0.305	0.154	0.267	0.114	0.482	0.088	0.505	0.081	0.520	0.083	0.548

Plate 3A - 317 min

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.077	0.083	0.091	0.075	0.078	0.077	0.077	0.077	0.077	0.078	0.077	0.079
B	1.551	1.832	1.483	1.821	1.711	2.315	1.046	2.029	0.594	1.496	0.199	1.258
C	1.566	1.919	1.294	1.892	1.272	2.130	0.698	1.958	0.334	1.468	0.138	1.286
D	1.523	1.734	1.136	1.844	0.906	1.873	0.454	1.705	0.236	1.345	0.102	1.192
E	1.454	1.613	0.831	1.357	0.607	1.646	0.290	1.575	0.173	1.245	0.089	1.251
F	1.299	1.328	0.605	1.054	0.393	1.468	0.194	1.422	0.134	1.191	0.083	1.286
G	1.027	0.953	0.391	0.746	0.244	1.247	0.141	1.164	0.101	1.147	0.082	1.275
H	0.716	0.757	0.300	0.623	0.193	1.159	0.116	1.205	0.095	1.231	0.081	1.211

Signed Heidi Sleister Date

Witnessed Teresa Thompson Date

contd - plate B

3B3 ... 4E12 4F12 Plate#1 ... 6F12 Date ... 55L

	1 (3B3) metlo	2 wt	3 (4E12) metlo	4 wt	5 metlo	6 wt	7 metlo	8 wt	9 metlo	10 wt	11 metlo	12 wt	
A	0.072	0.073	0.072	0.072	0.075	0.072	0.072	0.075	0.072	0.072	0.072	0.071	← Blank row A
B	0.084	0.212	0.091	0.261	0.096	0.219	0.084	0.243	0.221	0.326	0.217	0.261	L1
C	0.078	0.202	0.083	0.238	0.080	0.226	0.078	0.240	0.182	0.300	0.167	0.215	2.58 mM
D	0.073	0.190	0.076	0.253	0.077	0.197	0.077	0.230	0.141	0.273	0.139	0.197	Plat 3:5 — 1.258 — .638
E	0.072	0.201	0.074	0.218	0.077	0.190	0.075	0.201	0.113	0.260	0.114	0.178	.318
F	0.071	0.164	0.072	0.189	0.073	0.170	0.072	0.190	0.086	0.212	0.093	0.135	.168
G	0.071	0.197	0.072	0.198	0.074	0.168	0.072	0.177	0.086	0.196	0.082	0.114	.088
H	0.071	0.179	0.072	0.172	0.073	0.164	0.072	0.167	0.079	0.199	0.079	0.094	.048

Plt 3B-23 min

Formula: L1

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.073	0.076	0.073	0.073	0.078	0.072	0.073	0.085	0.073	0.072	0.072	0.072
B	0.096	0.358	0.111	0.465	0.115	0.344	0.100	0.388	0.363	0.545	0.316	0.390
C	0.088	0.333	0.094	0.433	0.088	0.347	0.087	0.377	0.278	0.492	0.241	0.339
D	0.081	0.308	0.083	0.403	0.085	0.321	0.083	0.356	0.204	0.442	0.186	0.315
E	0.075	0.340	0.077	0.354	0.081	0.291	0.078	0.336	0.151	0.388	0.142	0.248
F	0.073	0.299	0.074	0.302	0.075	0.278	0.074	0.316	0.104	0.347	0.113	0.183
G	0.073	0.325	0.073	0.325	0.076	0.269	0.073	0.274	0.097	0.312	0.092	0.141
H	0.072	0.328	0.073	0.267	0.075	0.247	0.073	0.263	0.085	0.300	0.089	0.111

L1 — Plat 4:1 — Plt 3B-40 min

Formula: L1

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.074	0.077	0.075	0.074	0.077	0.074	0.074	0.074	0.073	0.073	0.073	0.073
B	0.118	0.494	0.135	0.615	0.135	0.464	0.112	0.511	0.487	0.744	0.435	0.529
C	0.097	0.462	0.104	0.554	0.097	0.458	0.093	0.488	0.360	0.666	0.323	0.460
D	0.084	0.439	0.089	0.528	0.091	0.416	0.088	0.470	0.259	0.611	0.246	0.410
E	0.079	0.454	0.080	0.464	0.091	0.380	0.082	0.446	0.189	0.545	0.178	0.322
F	0.075	0.435	0.076	0.416	0.079	0.378	0.076	0.421	0.120	0.473	0.136	0.240
G	0.075	0.439	0.075	0.422	0.078	0.341	0.074	0.359	0.109	0.416	0.104	0.175
H	0.074	0.441	0.075	0.384	0.077	0.331	0.074	0.351	0.092	0.397	0.095	0.133

L1 — Plat 4:4 — Plt. 3B-70 min

Formula: L1

Signed Heidi Sleister Date

Witnessed Teresa Thompson Date

Date

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.075	0.077	0.076	0.075	0.078	0.075	0.075	0.074	0.073	0.074	0.074	0.074
B	0.130	0.614	0.159	0.785	0.156	0.609	0.130	0.679	0.673	1.031	0.583	0.721
C	0.103	0.595	0.117	0.717	0.107	0.583	0.101	0.655	0.485	0.925	0.431	0.618
D	0.090	0.569	0.102	0.707	0.100	0.538	0.095	0.636	0.342	0.840	0.326	0.556
E	0.082	0.607	0.085	0.621	0.101	0.512	0.086	0.607	0.240	0.747	0.226	0.427
F	0.077	0.578	0.078	0.561	0.082	0.506	0.078	0.555	0.141	0.650	0.166	0.313
G	0.077	0.563	0.077	0.528	0.081	0.430	0.077	0.475	0.123	0.565	0.119	0.214
H	0.075	0.555	0.077	0.512	0.079	0.431	0.076	0.461	0.100	0.517	0.103	0.159

Formula: L1

• L1 ʌʌ Plat 5:2

Hmo

Pl+3B-110 min

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.082	0.089	0.084	0.082	0.088	0.081	0.081	0.081	0.077	0.081	0.078	0.080
B	0.228	1.509	0.319	1.911	0.300	1.437	0.228	1.595	1.551	2.402	1.385	1.719
C	0.160	1.370	0.194	1.748	0.166	1.402	0.153	1.520	1.114	2.149	1.004	1.469
D	0.125	1.332	0.144	1.651	0.150	1.287	0.133	1.475	0.760	1.948	0.712	1.279
E	0.101	1.388	0.109	1.437	0.139	1.165	0.106	1.381	0.508	1.745	0.470	0.968
F	0.090	1.302	0.092	1.288	0.102	1.129	0.092	1.298	0.258	1.483	0.324	0.678
G	0.087	1.301	0.088	1.227	0.098	0.992	0.087	1.074	0.209	1.289	0.196	0.451
H	0.084	1.303	0.086	1.133	0.092	0.947	0.084	1.039	0.144	1.160	0.145	0.289

Formula: L1

• L1 ʌʌ Plat 8:4

Hmo

Pl+3B-316 min

Signed Heidi Meister Date Witnessed Teresa Thompson Date

Data from notebook #3295, p. 52-55

7C10

Minutes	7C10 met10	wt
21	0.149	0.164
45	0.308	0.312
75	0.43	0.439
110	0.487	0.51
317	1.299	1.328

3B3

Minutes	met10	WT
21	0.084	0.212
45	0.096	0.358
75	0.118	0.494
110	0.13	0.614
317	0.228	1.509

3E3

Minutes	met10	WT
21	0.172	0.239
45	0.281	0.423
75	0.379	0.589
110	0.472	0.749
317	1.136	1.844

4E12

Minutes	met10	WT
21	0.091	0.261
45	0.111	0.465
75	0.135	0.615
110	0.159	0.785
317	0.319	1.911

6F12

Minutes	met10	WT
21	0.148	0.237
45	0.22	0.407
75	0.3	0.581
110	0.387	0.762
317	0.906	1.873

4F12

Minutes	met10	WT
21	0.096	0.219
45	0.115	0.344
75	0.135	0.464
110	0.156	0.609
317	0.3	1.437

1B6

Minutes	met10	WT
21	0.103	0.229
45	0.13	0.378
75	0.173	0.532
110	0.211	0.716
317	0.454	1.705

5C5

Minutes	met10	WT
21	0.084	0.243
45	0.1	0.388
75	0.112	0.511
110	0.13	0.679
317	0.228	1.595

4F10

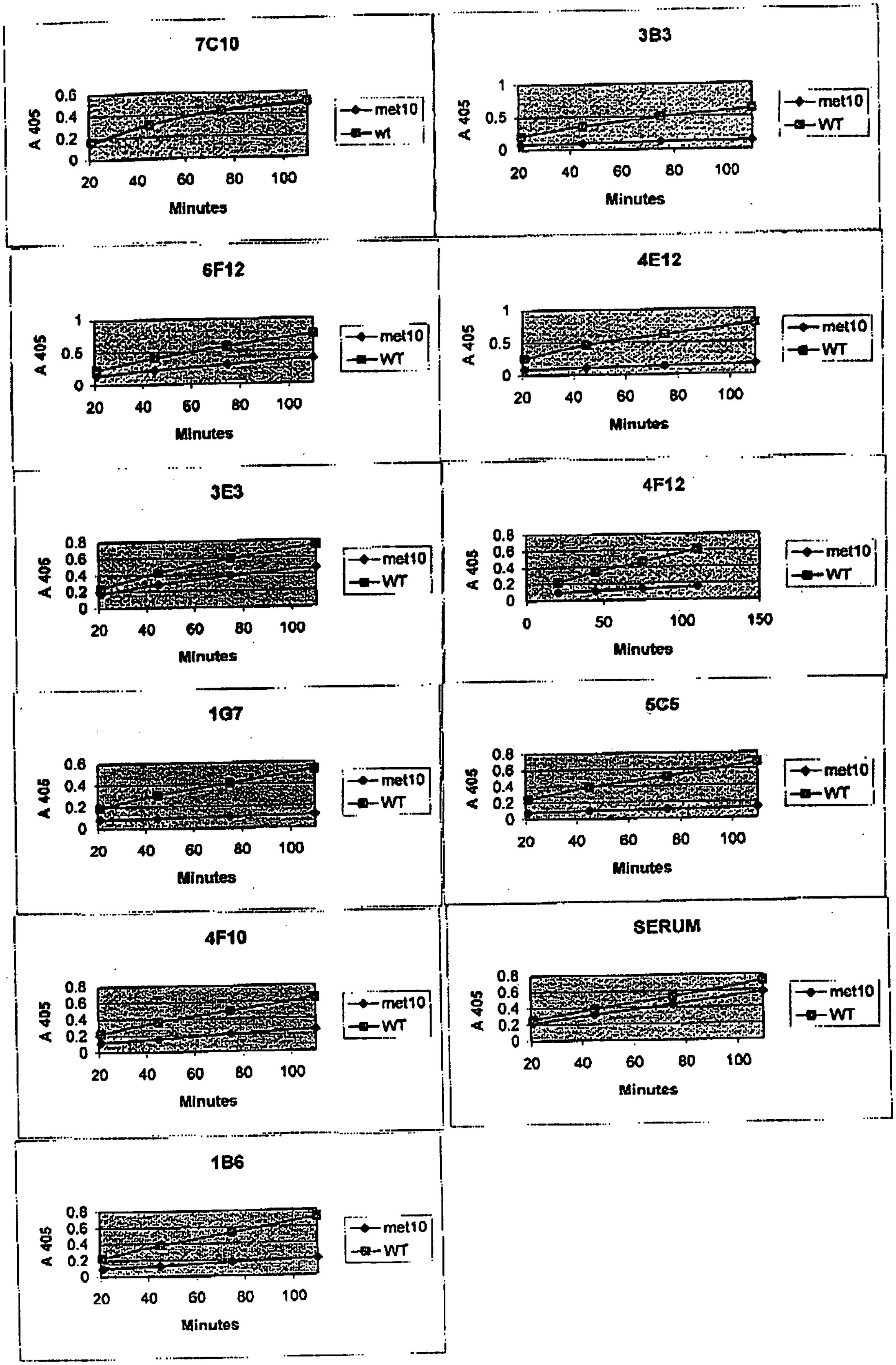
Minutes	met10	WT
21	0.118	0.223
45	0.158	0.364
75	0.21	0.491
110	0.258	0.642
317	0.594	1.496

SERUM

Minutes	met10	WT
21	0.217	0.261
45	0.316	0.39
75	0.435	0.529
110	0.583	0.721
317	1.385	1.719

1G7

Minutes	met10	WT
21	0.083	0.191
45	0.091	0.305
75	0.104	0.413
110	0.116	0.536
317	0.199	1.258



Data from notebook #3295, pp. 52-55